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VOLUME 43 OCTOBER, 1953 NUMBER 10

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

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. . . THROUGH CHEMISTRY

STRIATE MOSAIC, A NEW DISEASE OF WHEAT IN SOUTH DAKOTA [1]

J. T. Slykhuis [2]

While surveying for wheat streak mosaic in the winter wheat areas of south central South Dakota in 1950-51, a hitherto unknown mosaic disease was found on wheat in 5 counties. This disease, named striate mosaic,[3] was found transmissible only by the leafhopper *Endria inimica* (Say) whereas wheat streak mosaic was transmissible manually. Striate mosaic was reported earlier by the author (4, 5) but data pertaining to its transmission by the vector were not presented.

SYMPTOMS.—The first symptoms of striate mosaic appear as faint chlorotic dashes or streaks along the veins, becoming visible most clearly on the lower surface of the leaves (Fig. 1). Necrosis usually follows chlorosis, and leaf destruction often becomes severe. Brown necrotic spots and streaks develop in certain varieties. Plants become stunted (Fig. 2) and yield partially sterile heads and lower quality grain. Some plants die before heads are formed.

[1] Accepted for publication June 16, 1953.

The author wishes to acknowledge the help and suggestions given by Dr. C. M. Nagel, Head, Plant Pathology Department, during the preparation of the manuscript.

Approved for publication by the Director of the South Dakota Agricultural Experiment Station as Journal Series No. 297.

[2] Assistant Plant Pathologist, Plant Pathology Department, South Dakota Agricultural Experiment Stations, Brookings, South Dakota.

[3] The name was suggested by H. H. McKinney when he was shown diseased plants by the author in June 1951.

FIG. 2. Stunting resulting from striate mosaic on Rushmore (A) and Minter (B) wheat. The plants in the right hand pot of each pair were infected with striate mosaic by *E. inimica*. Note that some plants died. Plants on the left were healthy.

LEAFHOPPER TRANSMISSION STUDIES. — Leafhopper transmission of this virus was first performed with leafhoppers collected from fields of mosaic-infected wheat in Gregory County and from fields of mosaic-free wheat in Brookings County. The principal species included *Aceratogallia sanguinolenta* (Prov.), *Endria inimica* (Say), *Exitianus exitiosus* (Uhler) (Stahl),

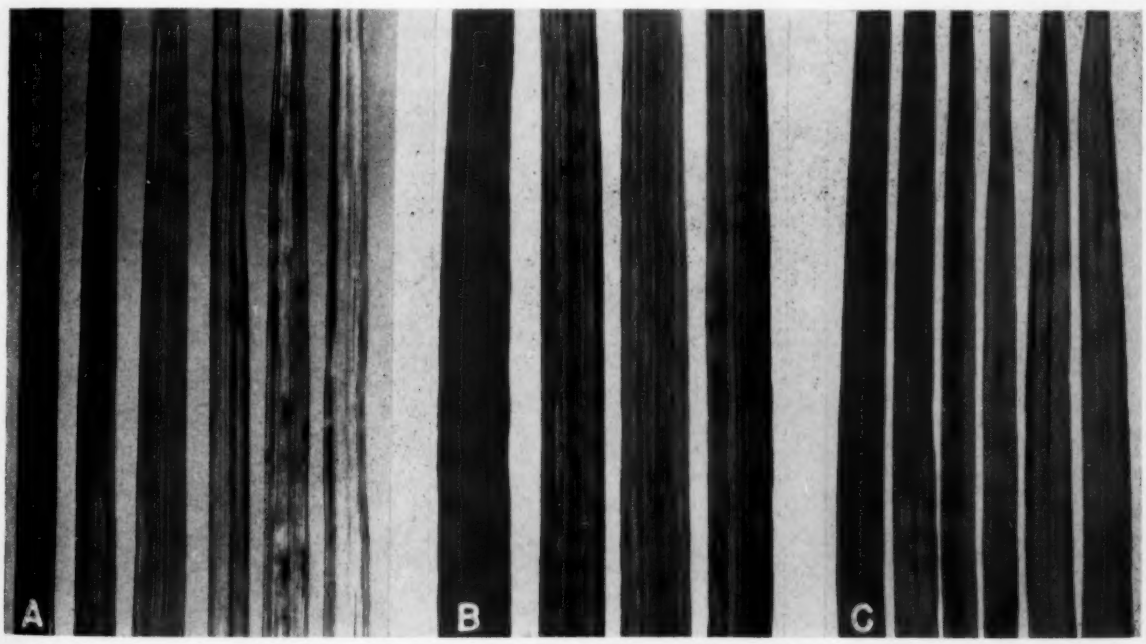

FIG. 1. Foliage symptoms of striate mosaic on 3 varieties of wheat; Minter (A), Exchange (B), and Mindum (C). In each case the leaves on the left are healthy; the others show symptoms varying from mild chlorotic dashes and streaks to severe chlorosis.

TABLE 1.—*Transmission of striate mosaic from diseased to healthy wheat by the leafhopper, E. inimica*

Stage of Leafhoppers	No. of lots of insects feeding on		No. of lots of insects transmitting virus from	
	Healthy Plants	Diseased Plants	Healthy Plants	Diseased Plants
3rd nymphal	0	3	0	1[a]
4th nymphal	1	6	0	4
5th nymphal	2	5	0	2
adult	3	8	0	5
nymphs and adults	5	7	0	5
Total of lots of insects	11	29	0	17

[a] The percentage of test plants that became diseased after the feeding of each colony varied from 20 to 100 per cent with an average of 67.3 per cent.

Macrosteles divisus (Uhler), and *Psammotettix striatus* (Linn.)[4] Ten insects of each of these species were caged 10 days on greenhouse, pot-grown, healthy Minter wheat seedlings enclosed in small cloth cages. Of these only *E. inimica* collected from fields of mosaic-infected wheat in Gregory County transmitted the virus. All species collected from fields of mosaic-free wheat in Brookings County failed to transmit the virus.

In a later study, leafhoppers collected from mosaic-free fields were confined 6–10 days on diseased or healthy greenhouse-grown plants and subsequently transferred and confined individually to healthy wheat plants for 7–10 days. Again only *E. inimica* of the tested species transmitted the virus from diseased plants, whereas it and the other species failed to transmit the virus from healthy plants.

In a further study, nymphs and adults of *E. inimica* were fed on diseased field plants transplanted to the greenhouse. All these stages of the insect transmitted the virus. (Table 1) Transmission failed in some of the lots presumably because in those instances streak mosaic-infested plants were mistakenly included for striate mosaic-infected plants as the virus source.

PERTINENT FACTORS IN THE TRANSMISSION OF STRIATE MOSAIC BY ENDRIA INMICA. —Adult *E. inimica* leafhoppers were collected from a blue grass field in a mosaic-free area. One group was confined on disease-free wheat for 2 days while other groups were confined 16 hours, 2 days, or 8 days on wheat diseased with striate mosaic. After these periods of confinement the leafhoppers were transferred individually to single healthy Minter wheat seedlings beneath 2.6 × 20 cm. cages of 32 mesh plastic screen (Fig. 3). The numbers of leafhoppers initially transferred from the source plants were: 15 after 2 days on the initial disease-free wheat, 13 after 16 hours, 6 after 2 days, and 70 after 8 days on diseased wheat. At intervals of 1-2 days at first and 4-11 days later, each leafhopper was transferred to new seedlings as long as the insect lived. All insects were dead by 88 days after the beginning of the experiment. The wheat test plants were examined frequently for symptoms of striate mosaic as long as 1½ months after the leafhoppers

[4] The species of leafhoppers were identified by Professor H. C. Severin, Head, Department of Entomology and Zoology, South Dakota State College.

FIG. 3. Types of cages used in transmission experiments with leafhoppers. A and B made from 32-mesh cotton cloth to confine a colony of leafhoppers on a number of plants. The double cage A allowed movement to leafhoppers between pots of plants. C shows cages used to confine individual leafhoppers on single plants. The later cages were made from a 32-mesh colorless plastic screen.

had fed on them.

Striate mosaic was not transmitted by any of the insects that had fed only on healthy plants, hence the leafhoppers used in the experiment were initially virus-free. Results from the insects confined for various periods on diseased plants indicated the following:

1) *Feeding time necessary for E. inimica to become viruliferous.*—Sixteen hours of confinement on diseased plants was ample for *E. inimica* to become viruliferous.

2) *Latent period of the virus in E. inimica.*—The shortest time lapse between first feeding on diseased plants and transmission of the virus was 10–14 days, and the longest was 31 days for insects caged 8 days on diseased plants. The latent period for all insects caged 16 hours and 2 days were within these extremes.

3) *Proportion of leafhoppers functioning as vectors.*—Only 33.7 per cent of all leafhoppers that had fed on diseased plants transmitted the virus, but many insects died before the end of the longest latent period. As the insect population declined with time, a higher proportion of the surviving population consisted of insects that had transmitted the virus to at least 1 test plan. By the end of the longest latent period of 31 days, 65.2 per cent of the surviving leafhoppers had functioned as vectors.

4) *Time interval during which leafhoppers remain viruliferous.*—One leafhopper transmitted the virus as late as 76 days after it was removed from the virus source. Some leafhoppers transmitted the virus only once during their lifetime, others transmitted it irregularly 2 or more times; still others transmitted it regularly to a series of plants.

5) *Length of virus incubation in wheat.*—Symptoms of striate mosaic appeared in from 15 to 45 days, but usually within 25–35 days at greenhouse temperatures which fluctuated between 65° and 80° with a mean about 68° F.

HOST RANGES OF STRIATE MOSAIC.—The winter wheat varieties Minter and Nebred, which are the 2 most widely grown varieties in South Dakota, proved highly susceptible to striate mosaic in the greenhouse and field. All common spring wheats so far tested also proved susceptible. Thirty diverse varieties planted in field plots in June, 1951, near a field of winter wheat, became naturally infected with striate mosaic, showing considerable variability in symptom expression. A number of additional varieties tested in the greenhouse also proved susceptible. Of these, Rushmore, Lee, and Exchange, (*Triticum vulgare* Vill.) developed clearly defined streaks over the leaf veins. Mindum, Capelli (*T. durum* Desf.), and Vernal (*T. dicoccum* Schubl.) developed less defined striate symptoms but more blotchy chlorosis that resembled streak mosaic. Hope, Henry, and Frontana (*T. vulgare* Vill.) at first developed typical striate mosaic symptoms, but later, brown necrotic speckling, with areas of necrosis characteristic of the advanced stages of the disease on Minter.

Chevron barley (*Hordeum vulgare* L.) and Clinton oats (*Avena sativa* L.) showed mild chlorotic dashes and streaks of striate mosaic after viruliferous *Endria inimica* had fed on them in the greenhouse. *Eragrostis cilianensis* (All.) Lutati and *Panicum capillare* L. were naturally infected in wheat fields in Gregory and Bennett counties. Striate mosaic was transmitted from these plants to wheat by *E. inimica.*

SEASONAL OCCURRENCE AND SPREAD OF STRIATE MOSAIC ON WHEAT.—In 1950 striate mosaic was recognized during late summer and fall in volunteer and early-sown winter wheat. By the following May additional plants in these fields showed symptoms before *E. inimica* appeared as nymphs. Since this insect apparently overwinters only in the egg stage (3), the appearance of additional diseased plants in the spring indicated that such plants had been infected in the fall and retained the virus over the winter.

Spring, summer, and fall spread of the virus in the field was tested on several wheat varieties planted at 3-week intervals in a field where the disease occurred. Striate mosaic symptoms appeared on a few plants in early June and reached a high incidence during July and early August. Although the disease continued to appear on new plants during September, the rate of spread diminished appreciably. A close relationship was evident between rapid spread of the disease and the abundance of *E. inimica.*

TABLE 2.—*A comparison of characteristics of striate mosaic, Russian wheat mosaic, and Siberian oat mosaic*

Characteristics of the Diseases	Wheat striate mosaic	Russian wheat mosaic	Siberian oat mosaic
"Veined type" of streaking prominent on lower side of leaf	+*	+	+
Phloem necrosis	−	+	+
Death or dwarfing of plants, reduction in grain yield and grade	+	+	+
Not transmitted by manual methods	+	+	+
Not transmitted by seed or soil	+	+	+
Virus acquired by nymph leafhoppers	+	+	+
Virus acquired by adult leafhoppers	+	−	−
Virus transmitted by both nymphs and adults	+	+	+
Shortest latent period in vector (days)	10–14	5	6
Approximate percentage leafhoppers acting as vectors	65.2	50	37
Virus pathogenic to both wheat and oats	+	+	+
Virus overwinters in vector	−	−	+
Virus overwinters in main host crop	+	+	−

* + indicates presence of; − indicates absence of the characteristics indicated.

SIMILARITY OF STRIATE MOSAIC TO TWO CEREAL VIRUS DISEASES IN THE U. S. S. R.—Striate mosaic of wheat in South Dakota appears similar to a wheat mosaic described for European U. S. S. R. and to an oat mosaic described for Siberia (1, 2, 7, 8, 9, 10, 11, 12, 13). Expressions of host symptoms and the relationship of the viruses to their respective insect vectors are similar in many respects (Table 2).

SUMMARY

A virus disease transmitted by the leafhopper *Endria inimica* (Say) was found on winter and late spring wheat in South Dakota in 1950-51. "Striate mosaic" is the name proposed for this disease because the symptoms include fine chlorotic streaks along the veins of leaves.

Nymphs and adults of this leafhopper function as vectors.

The latent period of the virus in the adult vector is long; the shortest found was between 10 and 14 days. Some individuals transmitted the virus only once while others transmitted it many times before they died. About 65 per cent of the surviving population had transmitted the virus at least once by the end of the longest latent period for any leafhopper tested.

The host range of the virus includes *Triticum vulgare*, *T. durum*, *T. dicoccum*, *Avena sativa*, *Hordeum vulgare*, *Eragrostis cilianensis*, and *Panicum capillare*.

The virus overwinters on winter wheat.

Striate mosaic on wheat in South Dakota has several characteristics in common with 2 virus diseases reported on wheat and oats in the U. S. S. R.

SOUTH DAKOTA AGRICULTURAL EXPERIMENT STATION
BROOKINGS, SOUTH DAKOTA

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HOST RANGE AND PROPERTIES OF BRASSICA NIGRA VIRUS [1]

Edward S. Sylvester [2]

Several aphid-transmissible viruses inducing mosaic diseases in crucifers have been described (1, 2, 3, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, 23, 25, 26, 27, 28, 29, 30). Although most of them have been differentiated from one another by host range and property studies, the recent trend has been to divide the aphid-borne crucifer viruses into the turnip virus 1 group and the cauliflower virus 1 group (31).

The turnip virus 1 group, which includes most of the known crucifer mosaics, is characterized by the following: 1) characteristic symptoms are coarse chlorotic mottling and leaf malformation; 2) symptom expression is favored by temperatures above 24°C.; 3) systemic infections are not limited to *Cruciferae;* 4) thermal inactivation is below 70°C. Contrawise in the cauliflower virus 1 group: 1) the characteristic symptom usually is persistent vein-clearing; 2) symptom expression is favored by temperatures below 24°C.; 3) systemic infections are limited to *Cruciferae;* 4) thermal inactivation is above 70°C.

Viruses of the turnip virus 1 group tested serologically have been shown to be active and related, whereas those of the cauliflower virus 1 group were reported to be inactive (10). Electron-micrographs of the turnip virus 1 group have shown flexuous rod-shaped particles (10, 23).

Although Takahashi (23) did not place the *Brassica nigra* virus in either group, the type and degree of symptom expression in cruciferous hosts at temperatures above 24°C., and electron-micrographs, indicate that it belongs to the turnip virus 1 group. However, since attempts to infect normally susceptible *Nicotiana* species failed, this grouping might be tentative. For confirmation, property studies and some additional host range trials were made.

MATERIALS AND METHODS.—The virus, obtained originally from W. N. Takahashi, has been maintained in smooth leaf mustard, var. Tendergreen, *Brassica juncea* Coss. In mechanical inoculation trials juice extracted from diseased plants was rubbed on carborundum-dusted test plants with a cotton swab. Inoculated leaves were later rinsed with tap water. Insect transmission trials were made by transferring aphids with a camel's hair brush. Acquisition feeding periods were either watched and timed, or the insects were allowed a limited access period on the diseased plant. Insect-inoculated plants were fumigated with nicotine before being placed in the greenhouse for incubation. All experiments were done in randomized replicated series.

RESULTS.—*Properties.*—a) *Thermal inactivation.*—Two ml. of crude infectious plant juice in thin-walled pyrex tubes were heated in a water bath for 10.5 minutes at the following temperatures (± 0.5°C.): 50°, 55°, 60°, 65°, and 70°C. In the first experiment of 5 replications, 5 plants per treatment, the results were: 50°, 7/25[3]; 55°, 7/25; 60°, 0/25; 65°, 1/25; 70°, 0/25. As the tubes were not completely submerged, the positive result at 65°C. may have been due to contamination by unheated juice along the exposed portion. Three additional replications, 10 plants per treatment, were made. In this series positive reactions occurred only at the 50° and 55° intervals, indicating that thermal inactivations occur between 56° and 60°C. with a 10 minute exposure.

b) *Dilution tolerance.*—Crude juice was diluted with distilled water until the following dilutions were obtained: undiluted, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Two experiments, each with 5 replications, 5 plants per treatment, were done. The combined results were: undiluted, 35/50; 10^{-1}, 13/50; 10^{-2}, 9/50; 10^{-3}, 3/50; and 10^{-4}, 1/50. Only 1 positive result was obtained with the 10^{-4} dilution, indicating that here the dilution tolerance was being approached.

c) *Longevity in vitro.*—In the *in vitro* tests, allowing 2 ml. quantities of crude juice in cotton-plugged tubes to stand at room temperature (approximately 22°C.), 2 series were done, each using 5 replications, 5 plants per treatment. In 1 experiment the intervals used and results were: 24 hour, 10/25; 48 hour, 12/25; 72 hour, 5/25; 96 hour, 2/25; and 120 hour, 0/25. In the second they were: 24 hour, 17/25; 72 hour, 1/25; 96 hour, 1/25; 120 hour, 0/25; and 144 hour, 0/25. The virus survived *in vitro* at room temperature for 96, but not 120 hours.

Results of the property tests indicated that the *Brassica nigra* virus belongs in the turnip virus 1 group.

Host range.—A limited host range within the genus *Brassica* was reported by Takahashi, and also the fact that *Nicotiana tabacum* L. and *N. glutinosa* L. were not susceptible (23). This latter was confirmed in tests by the author in which 25 plants of each species were inoculated, with negative results. Although most strains of the turnip virus 1 group induce either local or systemic reactions in these 2 species of *Solanaceae*, exceptions have been reported. Turnip mosaic of LeBeau and Walker (13) failed to infect *N. tabacum*, while mustard mosaic of Dale (3) and severe stock mosaic of Tompkins (28) did not infect *N. glutinosa*. Hence a member of the group which infects neither *N. tabacum* nor *N. glutinosa* is not improbable. Similarly, although most strains of the cauliflower virus 1 group usually fail to infect tobacco species, Chinese

[1] Accepted for publication June 22, 1953.

I wish to thank Mr. E. J. Ranft for his technical assistance in the laboratory and greenhouse.

[2] Assistant Professor of Entomology, University of California, Berkeley 4, California.

[3] In ratios listed numerator is number of plants infected, denominator number inoculated.

cabbage mosaic of Tompkins and Thomas is reported to produce local lesions on both (30).

For confirmation and elucidation of the specificity of host plant-vector reactions (22) additional experiments were done using insect and/or juice inoculation. Tests were confined to the following *Brassica* species or varieties[4]:

Mustards: *B. campestris* L. yellow mustard (= wild turnip or annual turnip rape). *B. campestris* L. var. *rapa* (L). Hartum. (= *B. rapa* L.), turnip, var. Purple Top White Globe. *B. chinensis* L. (= *B campestris* L. var. *chinensis* L.), Chinese cabbage, var. Wong Bok, Michihli, Pak Choi, and Gai Choi. *B. hirta* Moench (= B. *alba* (L.) Rabenh. = *Sinapis alba* L.), yellow seeded white mustard. *B. juncea* (L.) Coss., smooth leaf or Indian mustard, var. Tendergreen. *B. nigra* (L.) Koch (= *Sinapis nigra* L.), black mustard, var. Trieste. *B. pekinensis* (Lour.) Rupr. (= *B. pe-tsai* Bailey = *B. campestris* L. var. *pekinensis* (Lour.) Rupr., Chinese cabbage, var. Pe-Tsai.

Rapes: *B. napus* L. var. *biennis* (Schubl. and Mart.) Reichb., winter rape, var. Dwarf Essex. *B. napus* L. var. *napobrassica* (L.) Reichb. (= *B. campestris* L. var. *napobrassica* (L.) DC., rutabaga, var. American Purple Top.

Coles or Cabbages: *B. oleracea* L. var. *acephala* DC., kale, var. Jersey or Thousand Headed. *B. olerocea* L. var. *botrytis* L., cauliflower, var. February. *B. oleracea* L. var. *botrytis* L., sprouting broccoli, var. Italian Green. *B. oleracea* L. var. *capitata* L., cabbage, var. Winter Colma. *B. oleracea* L. var. *caulorapa* DC. (= var. *gongylodes* L.), kohlrabi, var. Early White Vienna. *B. oleracea* L. var. *gemmifera* Zenker, brussels sprouts. var. West Coast Half Dwarf.

A preliminary experiment tested the susceptibility to aphid inoculation of the 18 cruciferous species or varieties, using samples of 25 or 50 plants in replicated series, 5 or 10 plants per treatment. Sources of the virus were infected *B. juncea* plants. For inoculation, green peach aphids, *Myzus persicae* (Sulzer), were fasted for 1–2 hours, given an acquisition feeding of 15 seconds, and then a test feeding period of approximately 60 minutes. The plants were inoculated with single insects. Results indicated that none of the 6 varieties of *B. oleracea* was susceptible as indicated by absence of symptoms, nor was Pe-Tsai or rutabaga. All other species or varieties proved susceptible. Grouping the plants into susceptibility-percentage ranges, the results were: 2–8 per cent infected, turnip, Pak Choi, Michihli, and Wong Bok; 12–18 per cent infected, white mustard and black mustard; 24–28 per cent infected, yellow mustard, smooth leaf mustard, rape, and Gai Choi.

[4] The taxonomy of the crucifers is somewhat confused, but the terminology used follows that of Albina F. Musil, 1948. Distinguishing the species of *Brassica* by their seed. U.S.D.A. Misc. Pub. no. 643: 1-35; and R. Ruggles Gates. 1950. Genetics and taxonomy of the cultivated brassicas and their wild relative. Bul. Torr. Bot. Club 77: 19-28.

In further experimental work, brussels sprouts, kohlrabi, sprouting broccoli, and kale in the *B. oleracea* group; Wong Bok and Michihli varieties of the Chinese cabbage group; and white mustard were eliminated. Thus the rest of the trials were made using cauliflower, cabbage, yellow mustard, smooth leaf mustard, black mustard, Pak Choi, Gai Choi, Pe-Tsai, rape and rutabaga. Pe-Tsai was included to test again its susceptibility since the rest of the Chinese cabbage group was susceptible, and rutabaga was included to compare it further with rape.

In the next trial each plant was inoculated with the *Brassica nigra* virus by using 1 aphid per plant of either the green peach aphid, or the false cabbage aphid, *Rhopalosiphum pseudobrassicae* (Davis). The insects were given a preliminary fasting of 64–268 (mean 126.0) minutes, allowed an acquisition feeding of 12–18 (mean 15.06) seconds for the green peach aphid and 12–30 (mean 19.8) for the false cabbage aphid, then transferred singly to test plants for a test feeding period of 58–115 (mean 63.7) minutes. The time required to inoculate 5 plants (the number used per treatment in each replication) varied from 3–11 (mean 5.3) minutes. A total of 6.85 per cent of the false cabbage aphids were missing at the end of the test feeding period, compared to 15.2 per cent of the green peach aphids ($\chi^2 = 12.25$, $P = > .01$) indicating that the latter species was the more restless. Restlessness was associated somewhat with the species or variety of test plant. Black mustard, cauliflower, Pak Choi, rape, rutabaga, and cabbage were less favorable (21.4, 15.7, 14.3, 12.8, and 11.4 per cent respectively, of the insects missing at the end of the test feeding period), than yellow mustard, smooth leaf mustard, Pe-Tsai, or Gai Choi (7.1, 5.7, 4.3, and 2.9 per cent respectively, of insects missing) as far as vector restlessness was concerned.

A total of 7 replications made with 5 plants per treatment, resulted in so few infections (Table 1, experiment I), that the healthy plants from each series were reinoculated (experiment II), in a series of 6 replications with 5 plants per treatment. Series lacking the required number of plants, due to removal of infected ones, were equalized by adding from a noninoculated reserve stock. Individuals of the 2 aphid species were collected in vials and fasted in a refrigerator overnight. The next morning they were given an access feeding period of 3–7 minutes, transferred in groups of 3 to each test plant, and allowed a test feeding period of approximately 1 hour. The plants then were fumigated.

Results (Table 1) indicated that the 2 *B. oleracea* varieties, cabbage and cauliflower, were not susceptible. The 3 mustard species (yellow, black and smooth leaf) and Gai Choi were approximately equal in susceptibility, with a tendency for yellow mustard to be the most susceptible of all inoculated hosts (adjusted $\chi^2 = 3.102$, $P = > .10$ when comparing yellow mustard to any of the other 3 species). Yellow mustard was probably more susceptible to infection

TABLE 1.—*Results of trials to determine the relative susceptibility of 10 Brassica species or varieties to inoculation with the Brassica nigra virus by 2 aphid species, Myzus persicae (Sulz.) and Rhopalosiphum pseudobrassicae (Davis)*

Brassica species	Experiment I[a]		Experiment II[b]		Total
	M. persicae	*R. psuedo.*	*M. persicae*	*R. psuedo.*	
Cauliflower	0[c]	0	0[d]	0	0
Cabbage	0	0	0	0	0
Yellow mustard	6	4	28	23	61
Black mustard	6	1	25	15	47
Smooth leaf mustard	8	1	28	10	47
Rape	3	0	28	9	40
Rutabaga	0	0	7	2	9
Pak Choi	1	2	4	9	16
Pe Tsai	1	0	10	13	24
Gai Choi	9	0	23	15	47
Total	34	8	153	96	291

[a] In experiment I, plants were inoculated with single insects, using a 15 second (*Myzus*) or 20 second (*Rhopalosiphum*) acquisition feeding and approximately an hour test feeding period.
[b] In experiment II, plants were inoculated with groups of 3 insects using a 3–7 minute access feeding period and approximately an hour test feeding period.
[c] Numbers in the columns are plants infected out of 35 inoculated.
[d] Numbers in columns are plants infected out of 30 inoculated.

by insect inoculation than either rape (adjusted $\chi^2 = 6.48$, $P = > .02$), or rutabaga, while rape was more susceptible than rutabaga. Although symptoms were mild in rape, there did not appear to be much support for the statement by Takahashi (23) that mild symptoms in *B. napus* indicated low susceptibility. In the *B. chinensis-pekinensis* group, Pak Choi and Pe-Tsai were about equal in susceptibility (adjusted $\chi^2 = 1.44$, $P = < .20$), while Gai Choi was more susceptible than either Pak Choi or Pe-Tsai (Gai Choi compared to Pe-Tsai, adjusted $\chi^2 = 9.28$, $P = > .01$).

Generally the green peach aphid was a more efficient vector than the false cabbage aphid. However, the relative vector efficiency seemed to be dependent on the host plant inoculated. Superiority of the green peach aphid was best demonstrated on black mustard, smooth leaf mustard, rape, or Gai Choi. On yellow mustard, the 2 species of aphids were approximately equal and relatively high in efficiency in comparison to their performance on the Chinese cabbage hosts (except Gai Choi). In the Chinese cabbage group only on Gai Choi did the green peach aphid indicate some, but not a statistically supported, tendency to be superior in efficiency (adjusted $\chi^2 = 1.61$, $P = <.20$). On Pe-Tsai, the 2 aphid species, while relatively inefficient, were approximately equal in inoculating ability. On Pak Choi, the false cabbage aphid seemed to be the superior vector, but again the tendency was not enough to allow mathematical support (adjusted $\chi^2 = 1.66$, $P = <.20$). However, the superiority of the false cabbage aphid over the green peach aphid in inoculating Pak Choi also has been indicated in previous work (22).

The evidence would seem to support the hypothesis that, other factors being constant, relative vector efficiency among aphids is dependent upon the host plant inoculated.

For comparative purposes, 10 plants of each species or variety were juice inoculated. No infections were obtained in cabbage, cauliflower, or rutabaga. Infections were obtained in all black mustard, yellow mustard, smooth leaf mustard, Pak Choi, Pe-Tsai, Gai Choi, and rape plants inoculated. Results indicated that comparative susceptibility correlation between juice and insect inoculations was not high, since no susceptibility differential was obtained in juice inoculation trials, and 1 variety, rutabaga, while susceptible when insect inoculated (9/65), was not susceptible when juice inoculated (0/10 and 0/15).

Symptomatology.—Takahashi (23) mentioned the symptoms on the species he inoculated, specifically noting severe mosaic symptoms on *B. juncea* and mild mosaic symptoms on *B. chinensis* and *B. pekinensis*. On *B. napus* the symptoms noted were faint yellow flecks and breaks in the waxy leaf bloom. Local lesions followed by systemic mosaic were mentioned as symptoms in *B. nigra*. On *B. carinata*, a species not included in this study, the symptoms reported were small rings on inoculated leaves and later a systemic red veination on young leaves. The evidence indicated that symptoms were influenced by the genetic derivation of the polyploid species, *B. juncea, B. napus*, and *B. carinata*.

In the present work some variations of these symptoms were noted, but generally results were confirmatory. Symptoms were as follows:

Yellow mustard.—Initial veinclearing developed on subsequent leaves into a coarse mosaic pattern of high contrast. Stunting evident, but not severe. Some leaf distortion, with leaves tending to become spatulate rather than rounded.

Smooth leaf mustard.—Initial veinclearing or, in juice inoculation, necrotic local lesions in a small percentage of the plants (Fig. 1). Usually initial fine veinclearing was replaced on later leaves by diffuse veinclearing, which developed into a rather coarse mosaic mottle of medium to high contrast. In a small percentage of plants, initial veinclearing was necrotic, and plants died with systemic necrosis. Stunting moderate, with some leaf narrowing, but no rosetting of leaves.

Black mustard.—With aphid inoculation, usually

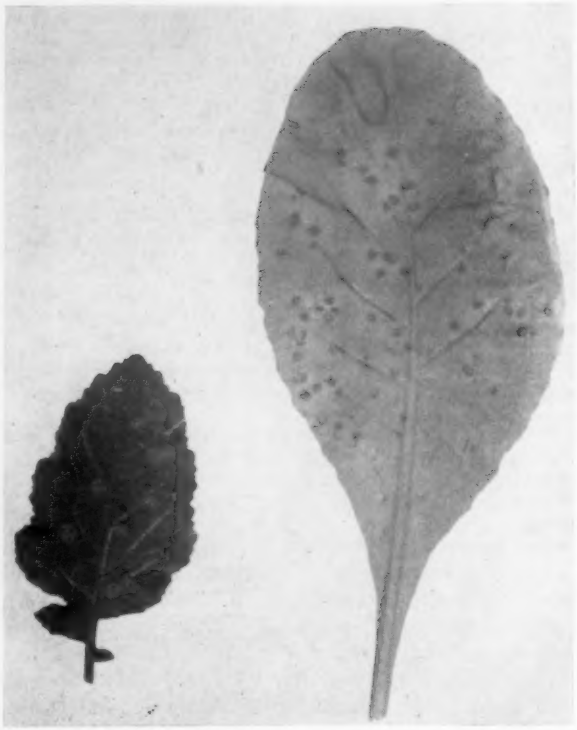

FIG. 1. Local lesions resulting from juice inoculation with the *Brassica nigra* virus, on *B. nigra* (left) and the polyploid species (*B. nigra* × *B. campestris*) *B. juncea* (*right*). The reaction occurred only occasionally in *B. juncea* (var. Tendergreen).

initial necrotic veinclearing, followed by systemic necrosis and death of plant. With juice inoculation, initial local lesions (Fig. 1) followed by systemic necrosis. If systemic necrosis was slow in developing, a finely mottled mosaic of high contrast appeared. Stunting severe with leaf distortion to point of rosetting. Premature leaf abscission, with axillary buds visible but leaves failed to emerge. Plants usually died rapidly, but some lived for 2 or more months.

The local and systemic necrotic reactions, occurring in nearly all of the *B. nigra* plants and a small percentage of the *B. juncea* plants, may indicate carrying over of the necrotic factor in *B. nigra* to a limited extent in the polyploid species *B. juncea* (23).

B. chinensis-pekinensis group.—In Pak Choi, initial veinclearing developed on subsequent leaves as an irregular coarse mosaic pattern of medium to high contrast. Stunting and leaf distortion were slight. On Gai Choi, symptoms were similar, but mosaic was not so coarse. In Pe-Tsai, initial symptoms were faint, diffuse veinclearing, replaced by diffuse, chlorotic flecking or spotting. Occasionally a low contrast mosaic mottle developed. Stunting was slight.

Rapes.—On both rape and rutabaga, initial faint, diffuse veinclearing was confined to larger veins. In rape, subsequent leaves developed faint chlorotic spots with breaks in the waxy leaf bloom. Spots were circular, 1–3 mm. in diameter, discrete or coalescent. Occasionally some spots became rings with darkened centers. At times a faint low contrast mosaic developed, without stunting or distortion. In rutabaga, after initial veinclearing, a medium contrast, coarse mosaic sometimes occurred with moderate stunting and distortion.

DISCUSSION.—Results of a literature survey of properties of 25 aphid-transmissible crucifer viruses are presented with those of *Brassica nigra* virus in Fig. 2. The base line is scaled in 2°C. intervals and represents the temperature of inactivation. Placement of viruses on the base line is relative since workers varied in the degree-intervals used, some working with 5°C. intervals, others with 2°C. Vertical height represents the highest dilution of the virus reported to give a positive reaction, while the diagonal designates hours *in vitro* survived. Again these 2 properties are somewhat relative since workers used various spacings in the hourly intervals with the several viruses. However, the diagram appears to be somewhat representative of actual differences which may exist among the various crucifer virus groups and strains. It can be seen that the *Brassica nigra* virus with an inactivation temperature in the 60°–61°C. interval, tolerance to a 10^{-4} dilution, and approximately 100 hour survival *in vitro*, fits well into the turnip virus 1 group (temperature of inactivation <70°C.). It is also of interest that the most variable feature of the strains of the cauliflower virus 1 group appears to be longevity *in vitro*.

The diagram suggests there may be a third group of aphid-transmissible crucifer viruses, for which the designation of the radish virus 1 group is proposed. This group, intermediate in respect to temperature of inactivation, is unusual in that its viruses occur in high concentrations in host plants, as evidenced by the high dilution tolerances, and are relatively stable, as indicated by the long survival time *in vitro*. The first virus described as radish mosaic by Tompkins (27) was reported to survive over 350 hours *in vitro*, while the one reported from Japan (5) survived over 500 hours. Further evidence that these 2 radish viruses may represent a distinct group may be deduced from a recent paper by Takahashi (24) in which particles from *B. juncea* plants infected with radish mosaic were shown to be short, rigid rods. The virus was originally obtained from C. M. Tompkins. The form of the particles associated with the cauliflower virus 1 group apparently has not been determined, but it is possible that they eventually will be found to be morphologically distinct from those of either the turnip virus 1 group or the radish virus 1 group. Also it would be of interest to learn whether or not members of the radish virus 1 group are serologically active, and if so, whether or not they would show similarities to the turnip virus 1 group, or be distinct.

In general, investigated vector-virus relationships for several of the crucifer viruses (3, 6, 7, 8, 9, 17, 18, 19, 20, 21, 22) indicate that the relationships are typical of the nonpersistent type (32), regardless of group. However, Kvicala (6) reported that the ornate

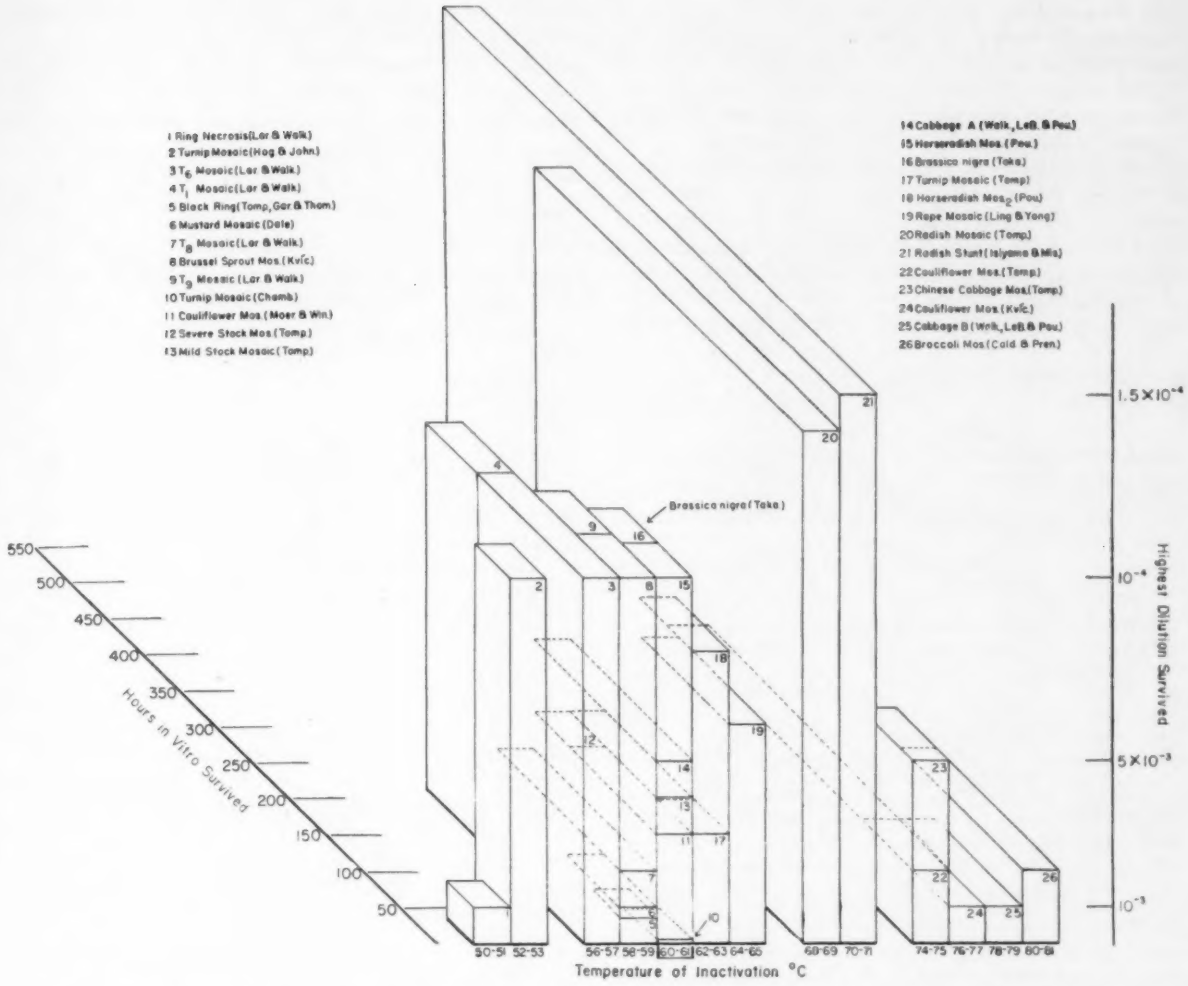

FIG. 2. Diagrammatic representation of the properties of 26 strains of crucifer viruses including the *Brassica nigra* virus. In addition to the 2 accepted groups of crucifer viruses, i.e., turnip virus 1 group (thermal inactivation <70°C.) and cauliflower virus 1 group (thermal inactivation >70°C.) there may be evidence of a third group (viruses 20 and 21), provisionally named the radish virus 1 group.

aphid, *Myzus ornatus* Laing, was a selective vector since it would transmit only a strain of the cauliflower virus 1 group from a plant infected with a strain of each group. This could be a special case concerned with a specific strain, or the vector might exhibit selectivity for the entire cauliflower virus 1 group. Attempts by the writer to transmit the *B. nigra* virus with *M. ornatus* have failed to date, adding some confirmatory evidence to the selectivity hypothesis of Kvicala. However, *M. ornatus* has been reported to transmit radish mosaic (2), and if this virus is a member of a distinct group, it would indicate that the aphid is not entirely selective. This fact might also indicate that the radish virus 1 group has some affinity to the cauliflower virus 1 group.

SUMMARY

Property studies with *Brassica nigra* virus in *B. juncea* indicate that thermal inactivation occurs at approximately 60°C. for 10 minutes, the dilution end point at about 10^{-4}, and longevity *in vitro* at room temperature is for 96 but not 120 hours.

Nicotiana tabacum or *N. glutinosa* were not susceptible to infection. None of 6 *B. oleracea* varieties tested was susceptible either by mechanical or insect inoculation. All mustards, *B. campestris* (yellow mustard), *B. campestris* var. *rapa* (turnip), *B. chinensis* (Chinese cabbage, var. Wong Bok, Michihli, Pak Choi, and Gai Choi), *B. pekinensis* (Chinese cabbage, var. Pe-Tsai), *B. nigra* (black mustard, var. Trieste), *B. juncea* (smooth leaf or Indian mustard, var. Tendergreen), and *B. hirta* (white mustard), were susceptible to insect inoculation and developed systemic mosaic symptoms varying in severity. Local lesions occurred on *B.* nigra and some individuals of *B. juncea* in juice inoculation trials. Two varieties of rape, *B. napus* var. *biennis* (winter rape, var. Dwarf Essex), and *B. napus* var. *napobrassica* (rutabaga, var. Ameri-

can Purple Top) were susceptible to insect inoculation, usually with mild symptoms. Rutabaga was not susceptible to mechanical inoculation in limited trials.

The relative efficiency with which 2 aphid species, *Myzus persicae*, the green peach aphid, and *Rhopalosiphum pseudobrassicae*, the false cabbage aphid, transmitted the virus seemed to depend on the species or variety of host plant inoculated. In general the green peach aphid was the better vector. Limited juice inoculation trials did not show any differences in susceptibility among the species inoculated, except in the case of rutabaga.

A diagrammatic grouping of the properties of 26 crucifer virus strains gave confirmatory evidence that the 2 accepted groups of crucifer viruses, i.e., the turnip virus 1 group and the cauliflower virus 1 group, can be differentiated on the basis of thermal inactivation temperatures. As to properties, possibly a third group of aphid-transmissible crucifer viruses exists, the first reported member of which was radish mosaic (27). A tentative designation of this group as radish virus 1 is proposed.

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INDUCED HYBRIDIZATION IN GRAMINICOLUS SMUT FUNGI. II. USTILAGO STRIIFORMIS × U. BULLATA [1]

George W. Fischer

During a study of the life history of the "*forma hordei*" of *Ustilago striiformis* (Westend.) Niessl (2) it was noticed that sporidia of this smut fungus would fuse readily with sporidia of opposite compatibility groups of *U. bullata* Berk., *U. hordei* (Pers.) Lagerh., and *U. avenae* (Pers.) Rostr. (*U. nigra* Tapke). This suggested that the stripe smut fungus might be induced to hybridize with the other 3 species if common hosts could be provided. In 1948, a brief report (3) was given of such hybridization obtained between *U. striiformis hordei* and *U. bullata*. Here is a more detailed account of the results obtained in crossing these 2 species.

The chief interest in a study of hybridization between *Ustilago striiformis hordei* and *U. bullata*, *U. hordei* and *U. avenae* lies in the inheritance of sorus characters. *U. striiformis* is characterized by the development of short to long, parallel, blackish sori in the leaves and sheaths of the host, thus accounting for the common name "stripe smut." Occasionally the sporulation will extend up the culm and into the rachis and glumes in the inflorescence. The other 3 smuts, being head smuts, sporulate almost exclusively in the inflorescence; only rarely does the sporulation extend downward to include the neck and/or flag leaf. The contrasting sorus characters, then, involved sporulation in vegetative tissues versus sporulation in reproductive tissues. Supplementing these 2 contrasting characters are also some spore characters. *U. striiformis hordei* has prominently echinulate spores of uniform color intensity and wall markings; spores of *U. bullata* are roughly verrucose and in some races they tend to have a darker equatorial band; spores of *U. hordei* are smooth and more or less lighter colored on 1 side; spores of *U. avenae* are minutely echinulate and also tend to be lighter colored on 1 side.

MATERIALS AND METHODS.—In a study of multiple compatibility groups in *Ustilago striiformis hordei*[2] (5), it was again found that sporidia of this variety of stripe smut would fuse readily with sporidia of *U. bullata*, provided of course, they represent intercompatible "sex" groups. It was further noted that some combinations of monosporidial cultures of these 2 smuts resulted in more profuse infection hyphae than did others. These apparently more compatible combinations were considered to be more promising of successful hybridization attempts and were therefore used as inoculum.

Cultures of the most compatible parent monosporidial lines were grown on plates of potato-dextrose malt-extract agar (2) to provide the basis for fresh, vigorous inoculum. The inoculum was prepared by placing approximately equal quantities of the parent monosporidial cultures on Petri dish plates of 3 per cent water-agar, mixing thoroughly and finally smearing the mixture evenly over the agar surface. The plates were incubated at room temperature until fusions had occurred and infection hyphae were abundant.

The host inoculations were performed in the same manner as described for inducing hybridization between *Ustilago hordei* and *U. bullata* (4).

Highly susceptible strains of 3 grasses were used which were known to be common hosts to *Ustilago striiformis hordei* and to 1 or more of the 3 inflorescence smuts involved in the hybridization attempts: *Agropyron trachycaulum* (Link) Malte, F67[3]; *Elymus canadensis* L., P2389, P2623; *E. glaucus* Buckl., P1851.

The parent cultures of *Ustilago bullata* and *U. striiformis hordei* were the same as those used in demonstrating inter-species compatibility from the standpoint of sporidial fusions (5). Thus the parent cultures of *U. bullata* represented 4 collections and 2 compatibility groups of that species. The parent cultures of *U. striiformis hordei* represented 12 collections and 6 compatibility groups (5). Each parent monosporidial culture was inoculated individually into the appropriate host plants to make certain that any alleged hybrids were not solopathogenic infections. Also, noninoculated check rows of each of the susceptible grass hosts were planted.

RESULTS.—*F_1 Sori.*—From a total of 63 parental combinations of *Ustilago striiformis hordei* and *U. bullata*, 18 F_1 hybrids were obtained. The infection percentages were comparatively low, ranging approximately from 5 to 35 per cent. No infection resulted in any instance from inoculation with individual parental monosporidial lines. Likewise, the check rows remained smut free. No sori resulted from the attempts to hybridize *U. striiformis hordei* with either *U. hordei* or *U. avenae*.

Without exception the F_1 sori were of the stripe type of the *Ustilago striiformis hordei* parent, but in most cases the sporulation was confined to the inflorescence. In this respect they resembled the *U. bullata* parent. The elongate sori were formed in the palea, lemma and proliferated flower parts, resulting in contorted and

[1] Accepted for publication June 19, 1953.
Scientific Paper No. 1222. Washington Agricultural Experiment Stations, Pullman, Wash. Project No. 451.

[2] This trinomial was suggested by Fischer and Shaw (6) for the biotypes of *Ustilago striiformis* parasitizing grasses of the tribe Hordeae.

[3] This refers to the author's own accessions; accession numbers with the prefix "P" are those of the Pullman Nursery Unit of Soil Conservation Service, Section of Nurseries, United States Department of Agriculture.

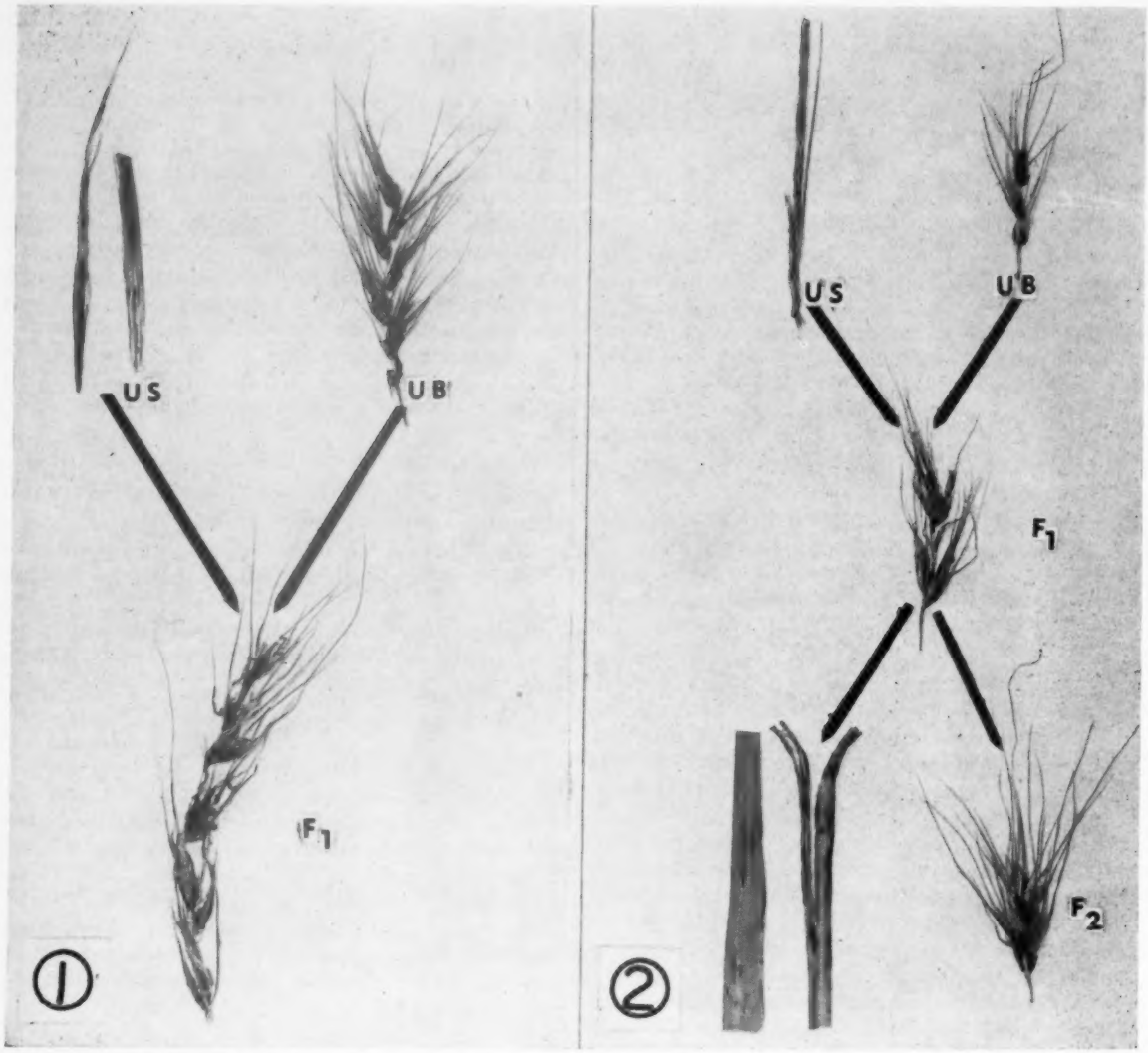

FIG. 1-2. Fig. 1. Hybridization between *Ustilago striiformis hordei* and *U. bullata*: Top, parents; bottom, F_1 showing stripe type sori in proliferated floral structures; host, *Elymus canadensis*. This particular hybrid (no. 740) failed to produce a second generation. × about ½. Fig. 2. Hybridization between *Ustilago striiformis hordei* and *U. bullata*: Hybrid 734 (see Table 1), showing parents, F_1 and F_2 generations, all on *Elymus canadensis*. Note stripe type sori in proliferated floral structures in the F_1 and that segregation in the F_2 is limited to parental types. × about ½.

twisted inflorescences especially in *Elymus canadensis* (Fig. 1).

F_1 spores.—In all the 18 hybrids, the spores were echinulate and uniformly pigmented, thus indicating dominance of the *Ustilago striiformis* microscopic characters as well as the general sorus type of that species.

Germination of the F_1 spores was readily obtained on potato-dextrose agar with 2 per cent dehydrated malt-extract broth added. Spore germination is quite different in the parents and thus affords an additional heritable character for possible study. *U. bullata* develops a simple definite promycelium, with sporidia borne at the crosswalls (1), whereas *U. striiformis hordei* produces a much branched, indefinite promycelium with sporidia sparsely and indiscriminately distributed over it (2). Although in most cases 50 to 60 per cent germination resulted, the type of germination did not resemble either parent. The promycelia were elongate, curved or slightly coiled, often with swollen tips. In many instances the promycelia burst at these swollen areas. Only a limited development took place in the others and then they gradually underwent lysis. Sometimes a few sporidia formed but they likewise did not survive. This is the same result described in the account of hybridization of *U. hordei* and *U. bullata* (4) and in similar work cited therein.

F_2 sori.—Since no sporidial cultures were obtained from germinating F_1 spores, it was necessary to use spores as inoculum to obtain F_2 sori. In general, the grass host upon which F_1 sori developed was also used as the host for the F_2 generation. The inoculated

TABLE 1. *Analysis of sorus and spore characters in F_1 and F_2 generations of hybrids between Ustilago striiformis hordei and U. bullata* [a]

Hybrid No.	F_1 Sorus Type	F_1 Spores	F_2 Sorus Type	F_2 Spores
680[b]	stripe (in heads)	echinulate	2 bullate	verrucose
686	stripe (in heads)	echinulate		
690	stripe (in heads)	echinulate	1 stripe	echinulate
692	stripe	echinulate	2 bullate	verrucose
694	stripe (in heads)	minutely echinulate		
697	stripe (in heads)	echinulate		
704	stripe (in heads)	echinulate		
711	stripe (in heads)	echinulate	3 bullate	verrucose
715	stripe	echinulate	12 stripe	echinulate
716	stripe (in heads)	echinulate	7 bullate, 1 stripe	verrucose, echinulate
718	stripe	echinulate	3 bullate	verrucose
720	stripe (in heads)	echinulate	2 bullate	verrucose
733	stripe (in heads)	echinulate	1 stripe	echinulate
734	stripe (in heads)	echinulate	7 bullate, 1 stripe	verrucose, echinulate
735	stripe	echinulate	5 bullate, 1 stripe	verrucose, echinulate
737	stripe (in heads)	echinulate	1 bullate	verrucose
738	stripe (in heads)	echinulate	3 bullate, 2 stripe	verrucose, echinulate
740	stripe (in heads)	echinulate		

[a] *U. striiformis hordei* is characterized by elongate, stripe-like sori, chiefly in the leaves, and by prominently echinulate spores; *U. bullata* is characterized by bullate sori, in the spikelets, and by verrucose spores.
[b] These numbers are merely taken conveniently from the greenhouse row number in which the F_1 occurred.

seed was planted in steam sterilized soil, and the resulting seedlings were transplanted to greenhouse benches where they grew to maturity.

Of the 18 F_1 hybrids, 13 gave rise to F_2 sori. The percentages of infection again were comparatively low, ranging from approximately 3 to 30 per cent.

Segregation evidently took place in the F_2 generation, but some of the segregates that normally might be expected to appear apparently were suppressed. Nine of the 13 F_2 hybrids both microscopically and macroscopically resembled 1 parent or the other only; 4 segregated into both parental types (Fig. 2) but none of the hybrids produced any segregates combining characteristics from both parents. Of the 9 hybrids which in the second generation resembled only 1 parent, 3 were strictly of the stripe type and had echinulate spores, thus strictly resembling the *Ustilago striiformis hordei* parent. The other 6 hybrids yielded F_2 sori of the bullate type, in the spikelets, and the spores were verrucose, thus strictly resembling the *U. bullata* parent. A summary analysis of the F_1 and F_2 generations of the 18 hybrids is in Table 1.

It is unusual that only strictly parental types should segregate from these hybrids. In every case of bullate sorus segregates, the spores were verrucose, often with a darker equatorial area. Likewise, in every instance of segregation of stripe-type sori, the spores were prominently echinulate and uniformly pigmented. It was hoped that intermediate segregates would appear which would combine some characters of both parents. Theoretically, these could be expected. However, none appeared in any of the 13 hybrids which developed a second generation. The study was not carried beyond the second generation.

These results, although disappointing from the standpoint of producing true hybrid lines combining certain characteristics of both smut parents concerned, do at least serve to show that *Ustilago striiformis hordei* and *U. bullata* will hybridize. Manifestations such as hybrid vigor and segregates unlike either parent, as were described in *U. hordei* × *U. bullata* hybrids (4), were lacking.

SUMMARY

Attempts were made to hybridize stripe smut, *Ustilago striiformis hordei*, with 3 inflorescence or head smuts, *U. bullata*, *U. hordei* and *U. avenae* (*U. nigra*) to study the inheritance of sorus and spore characters. Hybrids were obtained only between *U. striiformis hordei* and *U. bullata*. From a total of 63 parental monosporidial combinations of *U. striiformis hordei* and *U. bullata*, 18 F_1 hybrids resulted.

Without exception, the stripe-type of sorus was dominant in the F_1 generation although in most of the hybrids sporulation occurred in the palea, lemma and proliferated flower parts. Also without exception, the spores were echinulate and uniformly pigmented. Thus the characters of the *U. striiformis hordei* parent were dominant, with the exception of a strong tendency to sporulate in the heads.

Germination of the F_1 spores was readily obtained, but the promycelia and/or sporidia suffered so much lysis that cultures were not obtained. It was necessary, therefore, to use F_1 spores as inoculum to obtain the second generation.

The F_2 generation was obtained in 13 of the 18 hybrids. Segregation evidently occurred, but some of the segregates that normally might be expected apparently were suppressed. Nine of the 13 F_2 generations both microscopically and macroscopically resembled 1 parent or the other only; 4 segregated into both parental types, but none of the crosses produced segregates of true hybrid nature, that is, combining characteristics from both parents. All the stripe type segregates had

echinulate spores of uniform pigmentation, and all the bullate sorus types had verrucose spores with more or less darker equatorial pigmentation.

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THE EFFECTS OF AIR AND SOIL TEMPERATURES ON THE MULTIPLICATION OF TURNIP VIRUS 1 IN CERTAIN NICOTIANA SPECIES [1]

Glenn S. Pound and Lewis G. Weathers

In earlier studies the cabbage A and cabbage black ring strains of turnip virus 1 were shown to occur in cabbage in much greater concentration in plants growing at 28° C. than in plants growing at 16° (3). A gradient of virus concentration occurred, increasing from low to high temperature and corresponding closely with a similar gradient of symptom expression. In a later study (5) of horseradish mosaic, incited by another strain of this virus, the gradient of symptom response of horseradish to temperature was the reverse of that found in cabbage; symptoms were most severe at 16° and least severe at 28°. Here again assays showed a gradient of virus concentration that corresponded closely with that of symptom expression.

Since the horseradish strain did not readily infect cabbage and since no virus-free horseradish plants were available to use with the cabbage strains, all 3 strains were inoculated to certain species of *Nicotiana* where air temperature effects were studied. The cabbage A strain was also studied in detail in soil temperature experiments.

MATERIALS AND METHODS.—All hosts being studied for their response to infection were grown in white quartz sand in earthenware crocks, 7 plants per crock. They were watered at frequent intervals with a balanced nutrient solution to insure good growth, all crocks receiving identical amounts. Distilled water was added between feedings when needed. Plants were inoculated by the rubbing method and 1 crock for each host and each virus was placed immediately at each air temperature being studied.

In preparing inocula for concentration assays, a composite sample of 1 gm. of tissue made up from the youngest leaves of all plants was triturated in 10 cc. of distilled water or neutral phosphate buffer. For assays from inoculated leaves, a 3-gm. composite sample was used. The inocula were applied in half-leaf comparisons to tobacco (*Nicotiana tabacum* L. var. Connecticut Havana No. 38) on which the viruses produce only necrotic local lesions. Carborundum was blown on tobacco leaves with a DeVilbiss powder blower no. 119 prior to inoculation. Inocula were applied with cheese cloth pads. In all assays, the same incomplete block design previously described (5) was used. In the incomplete block design used, each inoculum was paired with each of the others in 3 half-leaf replications. Tests of significance were made by an analysis of variance of individual half-leaf comparisons. For simplication, however, the values presented in the tables are the overall averages of the 9 replications of each inoculum. The values given, therefore, do not always accurately show the difference that existed between individual comparisons but do show the concentration gradients.

The viruses used were the cabbage A strain (9), the cabbage black ring strain (8) and the horseradish strain HR1 (4), all previously shown by cross protection tests to be strains of turnip virus 1.

EXPERIMENTAL RESULTS.—*Air temperature.*— a) *Symptoms on Nicotiana glutinosa* L.—The responses to all 3 viruses showed similar temperature gradients with only minor differences among them. At 28° the cabbage black ring strain was the most severe at first; severe necrotic lesion development caused complete death of all inoculated leaves within 10 days. Stunting was very marked but only an occasional chlorotic or etched ring occurred on young systemically-infected leaves. As such leaves became older the rings or ringspots became more pronounced. Ultimately, the youngest leaves were completely symptomless. The cabbage A strain produced similar effects (Fig. 1) except less necrosis on inoculated leaves and less stunting. The horseradish strain produced only scattered chlorotic or etched rings on inoculated leaves and on systemically-invaded leaves

[1] Accepted for publication June 29, 1953.
Supported in part by a grant from the Wisconsin Alumni Research Foundation. Published with approval of the Director of the Wisconsin Agricultural Experiment Station. The present address of the junior author is Citrus Experiment Station, Riverside, California.

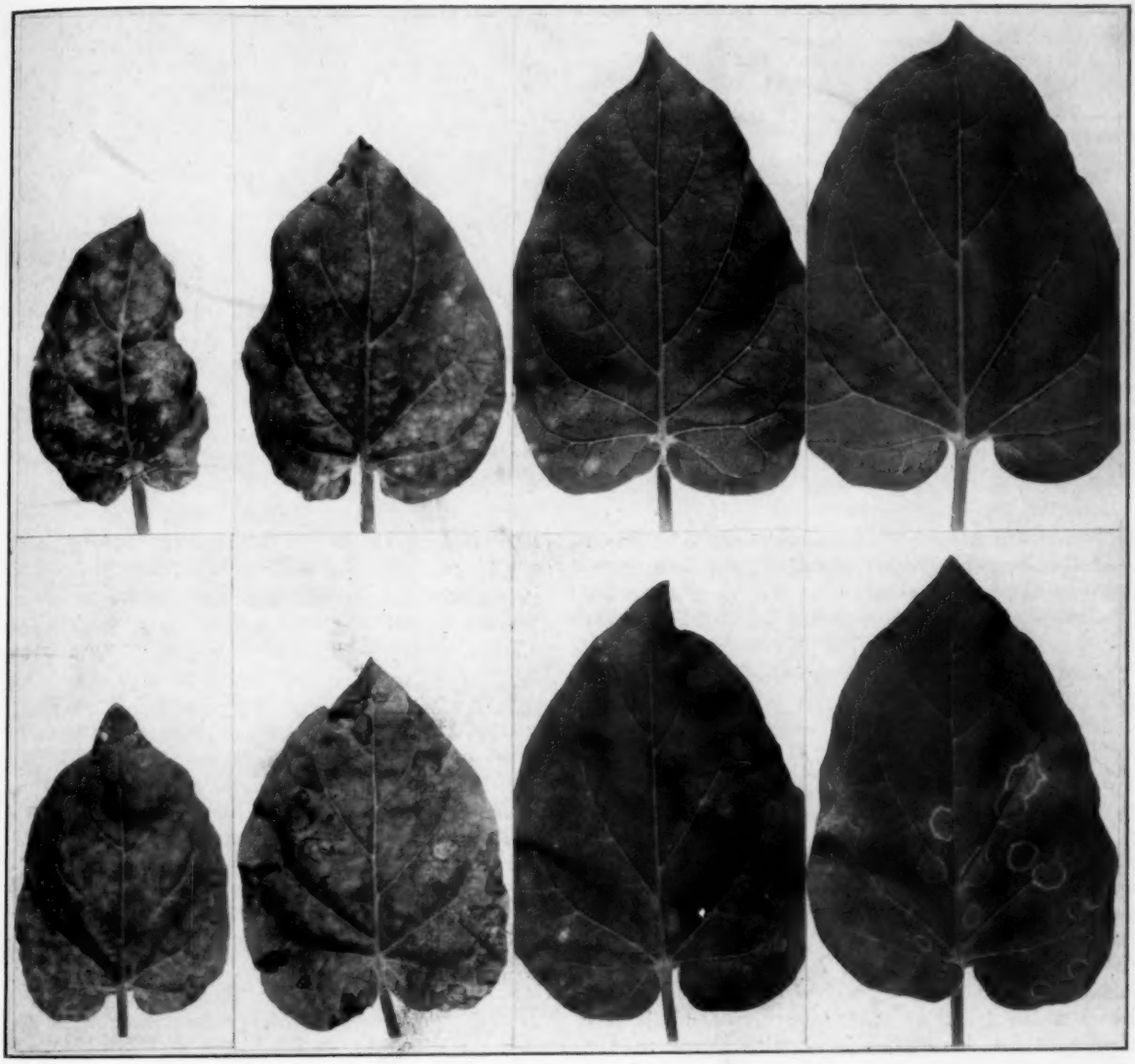

FIG. 1. Systemic symptoms produced on *Nicotiana glutino a* by cabbage virus A at 16°, 20°, 24°, 28° C. left to right, respectively. Above, youngest unfolded leaves 3 weeks after plants were inoculated. Below, leaves at intermediate position on plants at the same time.

when they became older. It was conspicuously the least severe.

At 24° all strains produced less severe symptoms on inoculated leaves than at 28° but systemic symptoms were more pronounced. These consisted of circular, well defined, chlorotic lesions with occasional etched rings as at 28°. No veinclearing or fine mottling occurred. At this temperature, the cabbage A strain was more severe than the cabbage black ring strain in stunting and in the prevalence of chlorotic lesions. Again, the horseradish strain induced the mildest disease and symptoms developed most slowly.

At 20° the cabbage black ring strain produced severe necrotic ringing on inoculated leaves and very severe stunting. Systemic symptoms consisted of slight veinclearing followed by a marked mosaic mottle. Symptoms were noticeably more severe than at 24° and 28°. The cabbage A strain showed less necrosis but more severe veinclearing, mottling, and stunting than did the cabbage black ring strain. The horseradish strain produced only circular chlorotic lesions, more pronounced than at 24° and 28° but much less severe than those with the other 2 strains. The incubation period of both cabbage strains was shorter at 20° than at any other temperature.

At 16°, symptoms of the cabbage black ring strain were similar to those produced at 20° but were slightly less severe. Those of the cabbage A strain were more severe than at 20° and on systemically-infected leaves showed more veinclearing, a fine diffuse mottle, and necrosis. This strain was obviously the most severe at this temperature. The horseradish strain

TABLE 1.—*The effect of air temperature on the multiplication of strains of turnip virus 1 in Nicotiana glutinosa*

Virus strain and source of inoculum	Assay interval (days after inoculation)	Ave.[a] number local lesions per half leaf with inoculum from plants grown at			
		16°C.	20°C.	24°C.	28°C.
Cabbage A					
Inoculated leaves	4	16	98	110	206
Tip leaves	7	1	47	38	29
Tip leaves	14	368	374	120	16
Tip leaves	28[b]	109	20	22	5
Cabbage Black Ring					
Inoculated leaves	4	14	183	215	103
Tip leaves	7	2	43	7	2
Tip leaves	14	93	71	12	0
Tip leaves	28[b]	32	66	9	2
HR1					
Tip leaves	14	84	16	0	0
Tip leaves	28[b]	23	21	1	0

[a] Values shown are averages of 9 replications. Decimals have been dropped.
[b] At the time of the 14 day assay temperatures were reversed. Values given from plants at 16°, 20°, 24°, 28° were grown before reversal at 28°, 24°, 20°, and 16° respectively. See text below.

also produced more severe symptoms at 16° than at any other temperature but was also much less severe and developed more slowly than the other 2 strains. The cabbage A strain ultimately was lethal only at 16°.

When temperatures were reversed, i.e. plants at 16°, 20°, 24°, 28° were transferred to 28°, 24° 20°, 16°, respectively, after 2 weeks a corresponding reversal in symptoms had occurred.

b) Virus multiplication in N. glutinosa.—Assays with the cabbage A strain showed that virus multiplication in inoculated leaves was most rapid at 28° and slowest at 16° with little difference between 20° and 24°. With the cabbage black ring strain the most rapid multiplication in inoculated leaves was at 24° and 20° and the slowest at 16°. Strain HR1 was not assayed from inoculated leaves. Each of the 2 cabbage strains showed most rapid invasion of tip leaves at 20°, with an incubation period of about 7 days. At 14 days after inoculation, however, the concentration from tip leaves was greatest at 16° and very low or almost nil at 28°. In some experiments the concentration at 20° was as great as at 16°. Strain HR1 was less invasive than the other 2 strains but the same temperature response occurred. Almost no virus was ever found in the tip leaves of plants infected with HR1 at 28° and 24°.

At the time of the 14-day assay, infected plants were moved from temperatures of 16°, 20°, 24°, 28° to temperatures of 28°, 24°, 20°, 16°, respectively. Two weeks after the reversals, assays were again made from the youngest tip leaves. It was found that with each strain a complete reversal of relative concentration, corresponding with the reversal in symptoms, had occurred. Plants grown at 28° before the reversal and in which systemic movement of the virus was greatly reduced, showed a much higher concentration after 14 days at 16° than plants moved from 16° to 28°. In some experiments with the black ring strain the highest concentration at 14 days after reversal was at 20° rather than at 16° but in assays made later than 14 days the concentration at 16° was greatest.

These experiments were repeated several times over a 4 year period. The data in Table 1 are from representative experiments.

Each virus strain at 28° produced very few or no symptoms on tip leaves. As these leaves matured, however, and came to be located at lower positions on the plant, they developed conspicuous ringspot lesions (Fig. 1). Inocula of the cabbage A strain taken from mottled tip leaves at 16°, from symptomless tip leaves at 28°, and from leaves at 28° showing ringspot lesions were compared in all possible half-leaf combinations. The average numbers of lesions per half leaf of the assay plant were 116.1, 0.4, and 66.0, respectively. This indicated that something in the metabolism of plants growing at 28° retarded the movement of the virus in the plant since it was shown that rapid multiplication would occur at this temperature when the virus was introduced in the leaves.

Plants of *N. glutinosa* infected with the cabbage A strain were grown under different levels of nutrition of nitrogen, phosphorus, potassium, and concentration of a balanced solution. The solutions and methods used were identical with those used by Cheo et al (2) except that in the present study the crocks were equipped with a siphon system which automatically drained them all to the same level.

In no instance (data not given) did nutrition alter the air temperature effect. Concentration of the virus in plants at 28° was very low, regardless of the nutritive treatment given the host. At 16° the concentration was very high compared to 28°, under all nutritive treatments.

At 16° effects of the various nutrition levels could be measured but at 28° multiplication was so low that differences in host nutrition were not measurable.

c) Virus multiplication in N. rustica L. and N. multivalis Lindl.—Only the cabbage A strain was tested in *N. rustica*. The virus did not invade tissue

TABLE 2.—*Effect of air temperature on multiplication of turnip virus 1 in Nicotiana rustica and N. multivalvis*

Virus strain and source of inoculum	Assay interval (days after inoculation)	Ave.[a] number local lesions per half leaf with inoculum from			
		16°C.	20°C.	24°C.	28°C.
Cabbage A in N. rustica					
Tip leaves	24	3	109	70	1
Cabbage A in N. multivalvis					
Inoculated leaves	4	10	17	44	63
Tip leaves	7[b]	239	525	469	448
Cabbage Black Ring in N. multivalvis					
Inoculated leaves	4	3	13	26	14
Tip leaves	7	205	211	233	246

[a] Values given are averages of 9 replications. Decimals have been dropped.

[b] Lesions in this assay were so numerous that accurate counting was difficult; hence, it is probably less accurate than other assays.

of this host as thoroughly as *N. glutinosa* and *N. multivalvis*. Hence, only the single assay was made. In Table 2 it is revealed that the virus multiplies in tip leaves in this host more at 20° than at 24°. Multiplication was very slight at 16° and 28°.

d) *Nicotiana multivalvis* yields a higher titre of turnip virus 1 in expressed sap than any other *Nicotiana* species tested. The cabbage A strain (Table 2) showed a rate of multiplication in inoculated leaves that increased with increase in temperature. The cabbage black ring strain showed the most rapid multiplication in inoculated leaves at 24°. In systemically-invaded leaves invasion was slightly slower at 16° than at the other temperatures but the ultimate production of both strains was about as great at one temperature as another. This corresponded with the symptoms which were similar at the 4 temperatures.

Soil Temperature.—Multiplication in N. glutinosa.—One experiment was performed in which the cabbage A strain was assayed from plants grown at an air temperature of 20–22° and at soil temperatures of 16°, 20°, 24°, 28°. The data (Table 3) showed that the most rapid multiplication was at 20° but that ultimately there existed a gradient of concentration which increased with decrease in soil temperature. Thus, soil temperature effects closely paralleled those of air temperature.

Since the virus and leaf tissue were at the same air temperature it can be reasoned that the soil temperature effects were indirect in nature and were not directly on the rate of reaction in leaves. The temperature effect on the root tissue must alter leaf metabolism which in turn affects either the rate of movement to the tip leaves or the rate of multiplication in the tip leaves.

Four experiments were run with the Cabbage A strain where plants grown at soil temperatures of 16° and 28° were tested at both 16° and 28° air temperatures. The 4 combinations (28° air–28° soil, 28° air–16° soil, 16° air–28° soil, 16° air–16° soil) were tested in half-leaf inoculations, in all possible combinations. Table 4 shows that, in general, soil temperature does not counteract the air temperature effects. It does, however, reduce the effect. For example, virus concentrations at 28° air–16° soil and 16° air–28° soil were generally greater than those at 28°–28° and 16°–16°, respectively. In all cases tip leaves of plants growing at 16° air temperature showed more virus than those from respective plants grown at 28° air temperature. This reaction was a reversible one as shown by the data of experiment 1.

Assays were made from the inoculated leaves in 2 experiments. One, at 5 days after inoculation, showed that multiplication at 28° air temperature was more rapid than that at 16° air temperature, irrespective of soil temperatures. The other, at 9 days after inoculation, showed that the concentration in leaves at 16° air–28° soil was much greater than in any other treatment. The same also was true in the assay from tip leaves made at 10 days. This indicates that following an initial period of incubation during which the virus multiplies most rapidly at high temperatures, further multiplication and movement of the virus in the plants occur most rapidly at 16° air–28° soil. This concentration in the tip leaves, however, may be due only to the rapidity of multiplication, and not to rapidity of movement. A similar shift was noted in the air temperature experiments where, following an initial period of most rapid multiplication at 28°, further multiplication and movement were most rapid at 20°.

An experiment similar to those shown in Table 4 was made with the cabbage black ring strain. Assays from tip leaves yielded data exactly similar to those in experiment 1 with the cabbage A strain.

TABLE 3.—*Effects of varying soil temperature on multiplication of cabbage virus A in N. glutinosa at an air temperature of 20-22°C.*

Source of inoculum and time of assay	Ave.[a] number local lesions per half leaf with inoculum from plants grown at			
	16°C.	20°C.	24°C.	28°C.
Tip leaves at 7 days after inoculation	8	155	114	28
Tip leaves at 14 days after inoculation	39	23	19	1

[a] Values given are averages of 9 replications. Decimals have been dropped.

TABLE 4.—*Effect of air-soil temperature combinations on multiplication of cabbage virus A in N. glutinosa*

Source of inoculum and time of assay	Ave.[a] number local lesions per half leaf with inoculum from plants grown at			
	28°air-28°soil	28°air-16°soil	16°air-28°soil	16°air-16°soil
Experiment 1				
Tip leaves at 19 days after inoculation	1	2	141	86
Tip leaves at 15 days after reversal[b]	7	8	82	75
Experiment 2				
Tip leaves at 10 days after inoculation	36	17	370	363
Tip leaves at 18 days after inoculation	9	5	65	38
Experiment 3				
Inoculated leaves at 9 days after inoculation	179	184	291	136
Tip leaves at 10 days after inoculation	1	17	297	45
Tip leaves at 21 days after inoculation	2	53	125	110
Experiment 4				
Inoculated leaves at 5 days after inoculation	112	127	45	36
Tip leaves at 11 days after inoculation	44	71	217	123
Tip leaves at 26 days after inoculation	1	32	100	107

[a] Values given are averages of 9 replications. Decimals have been dropped.

[b] At the time of the 19 day assay temperatures were reversed. Values given from plants at 28°air-28°soil, 28°air-16°soil, 16°air-28°soil, and 16°air-16°soil were grown before reversal, at 16°air-16°soil, 16°air-28°soil, 28°air-16°soil, 28°air-28°soil, respectively.

DISCUSSION AND SUMMARY

It is becoming increasingly evident that air temperature effects on virus multiplication in plants depend upon the specific host-virus complex, and that a virus does not always follow the same pattern. The cabbage strains of turnip virus 1 are known to occur in cabbage in greater concentration in plants grown at 28° than in plants grown at 16° (3). A horseradish strain of turnip virus 1 shows the exact reverse gradient in horseradish (5). Both of these hosts are so-called low temperature plants, the optimum air temperature for growth being about 20°. These 3 strains in *Nicotiana* species, all of which grow best at relatively high temperatures, show varied reactions. In *N. glutinosa* all 3 strains develop systemically much more rapidly and in greater concentration at 16° and 20° than at 24° and 28°. In fact, systemic movement and multiplication at 28° are practically nil. In inoculated leaves, however, the rate of multiplication increases with increase in temperature. This would seem to indicate that some product of metabolism of plants growing at 28° either inhibits movement of the virus or renders it noninfective. This reaction is being made the object of additional study. The air temperature reactions in *N. glutinosa* are completely reversible with reversal in temperature.

In *N.* multivalvis both cabbage strains multiply more rapidly in inoculated leaves at an air temperature of 28° than at 16° but the ultimate concentrations in systemically-invaded leaves vary little among the temperatures.

In *N. rustica* the cabbage A strain multiplies most rapidly and in greater concentration at an air temperature of 20°. At 16° and 28° systemic development is greatly retarded.

Soil temperature affects multiplication in *N. glutinosa* in much the same direction as does air temperature although to a lesser degree. Increasing the soil temperature at 16° air and reducing it at 28° air enhances multiplication over that where air and soil temperatures are the same. The soil temperature effects are also reversible reactions.

These reversible temperature reactions differ from temperature studies reported on effects of air and soil temperature in relation to cucumber virus 1 in spinach (1, 7) but are similar to studies of turnip virus 1 in cabbage (6).

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THIOURACIL INHIBITION OF TOBACCO MOSAIC VIRUS MULTIPLICATION [1]

Carl W. Nichols

Commoner and Mercer (1, 2) have reported that thiouracil at concentrations of 4.3×10^{-5} M or higher will completely inhibit tobacco mosaic virus (TMV) multiplication in tobacco leaf disks in continuous light (100 foot-candles) without causing death to the tissue. Partial reversal of this inhibition was achieved in the presence of an excess of uracil.

The purpose of this paper is to report the effect of light and dark on the inhibition of TMV multiplication by thiouracil and the effect of aqueous solutions of the chemical on TM infected tobacco plants growing in the greenhouse.

METHODS.—Thiouracil was tested against TMV multiplication in tobacco leaf disks floated on aqueous solutions of the chemical and in tobacco plants to which the chemical solutions were applied as sprays or as soil drenches. The leaf disks were obtained, floated, and analyzed by the "heat-ultra spectrophotometric" method described by Schlegel and Rawlins (3) with the exceptions that the samples were homogenized and diluted with 6 cc. of distilled water and the slow speed centrifugation steps were run at 11,500 × G for 10 minutes. Sixteen leaf disks were obtained from 24 hour inoculated plants for each sample. In each experiment, 3 samples were frozen immediately and the virus content determined (Table 1). Virus concentrations were determined from the absorbance-virus curve of Takahashi (5). In this paper these are expressed as mg. of virus per gm. of fresh tissue.

In certain experiments the effect of continuous light, continuous dark, and alternate light and dark on the inhibition of TMV multiplication by thiouracil in floated tobacco leaf disks was determined. In these experiments, 2 chambers were used in which the air was constantly pumped from one to the other so that the atmospheric conditions were the same in both chambers. One chamber was kept continuously at an illumination of about 115 foot-candles. The other was kept completely darkened. It was necessary for the samples being run in the dark to be exposed to diffuse light in the laboratory for about ½ hour a day while the solutions were being changed. In these tests distilled water checks were run in both chambers.

An indirect method was used to determine the amount of thiouracil that was taken up by the leaf disks over a given period of time.[2]

An aqueous solution of thiouracil has an absorption peak in the ultra violet range at a wave length of 275 mμ. Solutions of thiouracil held at room temperature in stoppered test tubes showed little or no change in optical density at 275 mμ during a 24 hour period. The amount of thiouracil absorbed by leaf disks was determined by obtaining the optical density of the thiouracil solutions before and after the leaf disks had floated on the solutions. The value obtained was corrected for changes in volume and for small amounts of ultra violet absorbing compounds which are secreted into distilled water by floated leaf disks.

TABLE 1.—*Virus recovered from tobacco leaf disks floated for 6 days in continuous light, continuous dark and alternate 24-hour periods of light and dark on thiouracil (100 mg./l.) and distilled water*

Treatment[a]	Mg. virus/gm. fresh tissue			
	Sample			
	1	2	3	av.
DW in continuous light	9.2	9.9	9.8	9.6
T in continuous light	0.39	0.27	0.51	0.39
DW in alternate light & dark	7.8	8.0	7.7	7.8
T in alternate light & dark	0.41	0.31	0.56	0.45
DW in continuous dark	4.1	4.1	4.3	4.2
T in continuous dark	2.1	1.9	2.3	2.1
Frozen immediately	0.31	0.28	0.38	0.32

[a] DW = distilled water; T = thiouracil (100 mg./l.).

Young tobacco plants were treated with various concentrations of thiouracil solutions by spraying with an atomizer, by soil drench applications, and by a combination of both methods. In the soil drench method all the liquid applied to the treated plants contained thiouracil in the concentration being tested and each plant was given as much liquid as it needed to supply normal water requirements. All plants were treated for 6 days with the chemical solution before they were inoculated. Just prior to inoculation, the lower leaves of all the plants were removed until all the plants had the same number of leaves. Each plant was inoculated on the 3 lowest remaining leaves by sprinkling lightly with carborundum, applying the juice from macerated TM infected tobacco leaves with a stiff brush and immediately washing the inoculated leaves. The chemical applications were continued for 10 days, at which time the plants were sampled. Samples were obtained from the inoculated leaves and from the smaller leaves. Eighteen disks (14 mm. in diameter), 6 from each of the inoculated leaves, and 1 small whole (minus midrib) leaf weighing about 100 mg. were obtained from each plant. Immediately after sampling, the fresh weights of the small leaves were determined. All samples were then frozen. These samples were analyzed for virus content in the same manner as the floated disks with the exception that only 3 cc. of distilled water were added to the homogenate of the small leaf samples.

RESULTS.—The results confirmed those of Commoner and Mercer (1, 2) who showed that solutions of thiouracil at very low concentrations can almost completely prevent TMV multiplication. In 1 typical test, TM infected leaf disks were floated on solutions of

[1] Accepted for publication June 24, 1953.

[2] This method was suggested by I. R. Schneider who has used it to determine the uptake of other chemicals by tobacco leaf disks.

thiouracil at concentrations of 1, 10, 100, and 500 mg/l. for 6 days in continuous light of about 115 foot-candles. The average amount of virus recovered from disks floated on a concentration of 1 mg/l. was 85 per cent of that recovered from those floated on distilled water; on 10 mg/l., 7 per cent; on 100 mg/l., 6 per cent; and on 500 mg/l., 8 per cent.

Tests were run to determine the effect of continuous light, continuous dark, and alternate 24 hour periods of light and dark on the effect of thiouracil on virus multiplication in tobacco leaf disks. The disks were obtained from 24-hour inoculated tobacco leaves and the experiment run for 6 days. For the alternate light and dark samples, the 24-hour light period was first. Results of 1 such experiment are in Table 1.

In disks floated on distilled water, the presence of light greatly stimulated the multiplication of TMV. In disks floated on thiouracil (100 mg./l) more virus was recovered from disks floated in the dark than from disks floated in the light. However, less virus was recovered from disks floated on thiouracil in the dark than on distilled water in the dark. In disks floated under conditions of alternate light and dark the results were much the same as those floated in continuous light with the exception that slightly less virus was recovered from the disks floated on distilled water in alternate light and dark than on distilled water in continuous light.

The presence of light greatly increased the ability of thiouracil (100 mg./l) to inhibit TMV multiplication (Table 1). It was thought possible that more water had evaporated from the samples in continuous light and thus produced a higher concentration of the chemical in these samples than in those in the dark. However, where the amount of liquid left from the original 10 cc. per sample after 24 hours was determined it was found that there was little difference in the amount of water that had evaporated from those petri dishes in the light as compared with those in the dark. In one test of 9 samples in the light and 9 in the dark, the amount of solution left in the former varied from 9.4 to 9.5 cc. and in the latter from 9.3 to 9.5 cc.

Another possibility was that less chemical moved into the leaf disks in the dark than in the light and the greater amount of virus present in the disks floated in the dark was due to the low concentration of chemical in the disks.

Tests were run to determine the amount of thiouracil that entered the leaf disks in light and dark by measuring the optical density of thiouracil solutions at 275 mμ. In 1 of these tests, leaf disks were floated on solutions of thiouracil at concentrations of 10 and 100 mg/l for 24 hours. Results (Table 2) showed that the presence or absence of light made little or no difference in the amount of chemical absorbed by tobacco leaf disks floated on a given concentration of thiouracil.

Sixteen leaf disks floated on 10 cc. of thiouracil at a concentration of 10 mg/l absorbed about 0.03 mg. of the chemical in 24 hours, and on a concentration of 100 mg./l about 0.15 mg. It is interesting to note that a 10-fold increase of concentration of the outside solution brought about a 5-fold increase of the chemical that went into the leaf disks. In either case in the light, the amount of recovered virus was reduced to about 5 per cent of that recovered from checks floated on distilled water.

TABLE 2.—*Mg. of thiouracil taken up in 24 hours by 16 tobacco leaf disks floated on 10 cc. of solutions at concentrations of 10 and 100 mg./l. under conditions of continuous light and continuous dark*

Treatment	Mg. thiouracil/16 disks			
	Samples			
	1	2	3	av.
10 mg./l. in continuous light	0.030	0.034	0.032	0.032
10 mg./l. in continuous dark	0.030	0.026	0.028	0.028
100 mg./l. in continuous light	0.159	0.154	0.134	0.149
100 mg./l. in continuous dark	0.157	0.157	0.158	0.157

When growing tobacco plants were treated with solutions of thiouracil, it was found that no combination of treatment and concentration of chemical could be used that inhibited virus multiplication without causing a moderate to severe chlorosis of some portion of the treated plant. The chlorosis appeared first on the younger leaves of the plant but, in some cases, also appeared on some of the more mature leaves.

The method of application where inhibition of virus multiplication was coupled with the least amount of injury was applying the chemical as a soil drench at a concentration 100 mg./l. When plants were treated for 16 days in this manner there was no apparent difference in size between the treated and check plants. Results of one such experiment (Table 3) show that it was possible to inhibit virus multiplication in both young and old leaves. However, the injury accompanying this inhibition was sufficient to make the method unsatisfactory as a control measure for TMV in tobacco.

Although the chlorosis induced by the chemical made it somewhat difficult to determine when symptoms occurred on the treated plants, it appeared to take at least 3 more days for symptoms to appear on the treated plants than on the checks.

DISCUSSION.—Takahashi (4) reported that light stimulated the multiplication of TMV in tobacco leaves. One possibility is that a higher temperature

TABLE 3.—*Virus recovered from TM infected tobacco plants watered daily with thiouracil (100 mg./l.) and distilled water*

Treatment and portion of plant assayed[a]	Mg. virus/gm. fresh tissue						
	Plant no.						
	1	2	3	4	5	6	av.
DW — YL	3.9	3.5	3.4	4.5	3.1	1.5	3.4
T — YL	0.75	1.9	0.55	0.57	0.06	0.27	0.65
DW — IL	2.3	1.9	2.3	2.5	3.1	2.6	2.3
T — IL	0.89	0.89	0.16	0.27	0.13	0.97	0.55

[a] DW = distilled water; T = thiouracil (100 mg./l.); YL = young leaves; IL = inoculated leaves.

occurs inside the leaves in light than in those kept in the dark, and a higher rate of virus multiplication takes place at this higher temperature. Yarwood (6) reported that the optimum temperature for TMV multiplication in tobacco is 31°C and that the rate of multiplication is less above and below this temperature. All of his virus assays were made within 24 hours after inoculation. In an experiment by I. R. Schneider and the author where tobacco leaf disks (obtained, inoculated, and floated according to the procedure followed in this paper) were floated in the dark at 25°, 28°, and 31°C, for 5 days, no significant difference in virus yield was obtained. It therefore appears that the effect of temperature observed by Yarwood does not continue long enough to be detectable 6 days after inoculation. However, more extensive studies must be made before conclusions are justified regarding the possibility that internal leaf temperatures may be involved in the stimulation of virus multiplication observed in the light.

The best known effect of light on plants is on carbohydrate metabolism. While it is not the only effect, it is one which should be considered in relation to thiouracil effects. Preliminary studies by David E. Schlegel of this department indicate that thiouracil has a marked effect on several phases of carbohydrate metabolism.

The following hypothesis is presented as a basis for further work in the field: in mosaic infected plant tissue growing in the light thiouracil inhibits a reaction usually involved in virus multiplication. In plants growing in the dark there is a breakdown of proteins and other components to compounds which may be utilized in virus formation, but the reactions involved in the use of these compounds are not inhibited by thiouracil.[3]

SUMMARY

Tobacco leaf disks infected with tobacco mosaic virus (TMV) were floated for 6 days in continuous light (115 foot-candles) on aqueous solutions of thiouracil, (1, 10, 100, 500 mg./l.) with no visible injury. Those floated on 1 mg./l. gave 85 per cent of the checks. Concentrations of 10 mg./l. and higher gave maximum inhibition (6-8 per cent of the checks).

Infected disks were floated for 6 days on thiouracil (100 mg./l.) and on distilled water in continuous light, alternate 24 hour periods of light and dark, and continuous dark. In the absence of thiouracil more virus was produced in the light than in the dark, but in the presence of thiouracil more virus was produced in the dark than in the light. More virus was produced on distilled water in the light than on distilled water in the dark.

Leaf disks absorbed thiouracil equally well in the dark and in the light and disks floated on thiouracil at a concentration of 100 mg./l. absorbed 5 times as much chemical as disks floated on a concentration of 10 mg./l. The strong inhibition of virus multiplication by thiouracil in the light was not due to greater absorption of chemical in the light.

No combination of method of application and concentration of chemical was found that would decrease virus multiplication in growing tobacco plants without causing injury to the plants. Applying thiouracil daily at a concentration of 100 mg./l. as a soil drench caused the least amount of injury while reducing virus recovered from both inoculated and young leaves to about 20 per cent of that recovered from distilled water treated checks. Symptoms appeared at least 3 days later on treated plants than on checks.

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[3] It has been suggested that the floating disk technic used may not be reliable because of the possibility that the metabolic products of bacteria in the solutions may take part in stimulation or inhibition of virus multiplication. As the method is used in this laboratory the solutions are discarded and the disks are washed in distilled water at 24 hour intervals to remove unattached bacteria and their metabolic products. In the testing of many compounds, done by several workers here, no correlation has been noted between bacterial growth and an effect on virus multiplication when the disks are washed at 24 hour intervals. The fact that thiouracil inhibits virus multiplication in growing plants (Table 3) appears to be conclusive evidence that thiouracil itself was the active agent.

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DIFFERENTIAL SURVIVAL AND NATURAL HYBRIDIZATION IN MIXED SPORE POPULATIONS OF TILLETIA CARIES AND T. FOETIDA [1]

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Tilletia caries (D.C.) Tul. and *T. foetida* (Wall.) Liro occur frequently in the same wheat field and occasionally on the same wheat plant (1,2). Different races of these species also may occur together in a similar manner. The predominance of 1 species over the other or of 1 race over others likewise is a common phenomenon (2,3,7). Ability to survive in competition with each other may be 1 of several factors contributing to the relative prevalence of these 2 species and their respective races in any given locality. A study therefore was undertaken to determine whether races of the bunt fungi have a differential capacity for survival in interspecies and inter-race mixtures. Observations also were made on the potentialities of natural hybridization under the conditions of this study. The results of these investigations are reported here.

MATERIALS AND METHODS.—Eleven physiologic races were used, including 9 of *Tilletia caries* and 2 of *T. foetida*. The races of the former species were T-1 through T-9, and those of the latter were L-2 and L-8 (7).

Chlamydospores of all races were collected from Ulka wheat (C.I. 11478) at Aberdeen, Idaho, in 1939. Spores of L-2 were mixed in equal proportions by weight with those of each of the 9 races of *T. caries*. Similar mixtures were made involving chlamydospores of L-8 and the *caries* races. Several separate mixtures of each of the desired combinations were made. Mixtures that were found by microscopic examination to have the 2 species present in approximately equal proportions, within an error of 5 per cent, were used in the tests. Seed of Ulka wheat, which is completely susceptible to each of the above races, was inoculated with the different spore mixtures and sown in duplicate 6-ft. rows at Aberdeen, Idaho, and at Bozeman, Mont.

When the wheat was in the hard dough stage, smutted heads were collected. In each row 50 smutted plants were selected at random and 1 head was harvested from each plant. The bunt balls from these 50 spikes were then threshed by hand, mixed, and crushed. The chlamydospores were examined microscopically to determine the relative percentages of the 2 species that survived passage through the host. The percentages were based on 5 random samples of 100 chlamydospores from each single-row composite. Each of these collections was used for a second test. The procedure was repeated in succeeding years. All spore lots were passed through Ulka at least 4 times, even though some apparently became pure after the third passage through the host.

RESULTS.—*Differential survival on the host.*—Data on relative survival of *Tilletia caries* and *T. foetida* in passage through Ulka wheat in successive years (1940-1946) at Aberdeen, Idaho, and at Bozeman, Mont., are presented as percentages of the latter species in Table 1. The variability in percentage of survival of a species in the duplicate rows at both Aberdeen and Bozeman was seldom greater than 10 per cent. Therefore, only the average percentage of survival for the duplicate rows is presented and each survival percentage is based on counts of 1,000 spores.

There were marked differences in the survival of races in the species mixtures. At Aberdeen, Idaho, L-2 either greatly predominated or eliminated the "T" races in 7 of the 9 mixtures with it. These 7 were T-2, T-3, T-4, T-5, T-6, T-7, T-9. Similar results were obtained at Bozeman, Mont., with these same race mixtures except the one incorporating T-7. Likewise, L-8 greatly predominated or eliminated the "T" races (T-2, T-5, T-6, T-9) in 4 of the 9 mixtures with it at Aberdeen, and in these same mixtures plus the 1 with T-3 at Bozeman. In no mixture was complete dominance of L-2 or of L-8 obtained in a single passage through the host. Apparently the number of passages required for complete elimination of 1 race from a mixture differed with the particular race of *T. caries* in the mixture. For example, T-2 was eliminated from the mixtures with L-2 and with L-8 during the third passage through the host at both Aberdeen and Bozeman. On the other hand the elimination process was more gradual with T-6. At Aberdeen this race was present in the mixtures with both L-2 and L-8 until the seventh passage. At Bozeman T-6 was eliminated from the mixture with L-2 in the fifth passage, while in the L-8 mixture 1.4 per cent of T-6 spores remained after the seventh passage.

Only 1 race of *Tilletia caries*, namely T-1, completely eliminated 1 of the *T. foetida* races. L-2 was eliminated from the original mixture in the seventh passage through the host at both Aberdeen and Bozeman. In contrast, 3 of the "T" races greatly predominated in combination with L-8. These were T-1 at Aberdeen and Bozeman, and T-4 and T-7 at Bozeman only.

Several of the species mixtures were recovered in approximately their original proportions after re-

[1] Accepted for publication June 24, 1953.

Cooperative investigations of the U. S. Dept. of Agr., Agr. Res. Admin., Bureau of Plant Industry, Soils, and Agricultural Engineering, Division of Cereal Crops and Diseases, and the Idaho, Montana, and Washington Experiment Stations. Scientific Paper No. 1189, Washington Agricultural Experiment Station, Pullman, Wash., Project No. 183; Journal Series Paper 298, Montana Agricultural Experiment Station, Bozeman, Mont.

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TABLE 1.—*Percentage survival of physiologic race L-2 and L-8 of Tilletia foetida in mixtures with nine different races of T. caries at Aberdeen, Idaho, and Bozeman, Mont., 1940-1946*

Original composite	Percentage survival of Tilletia foetida at Aberdeen, Idaho							Bozeman, Montana						
	1940	1941	1942	1943	1944	1945	1946	1940	1941	1942	1943	1944	1945	1946
L2 + T1	26.7	21.6	15.9	15.6	5.2	0.1	0.0	39.4	30.7	26.3	7.3	3.1	3.0	0.0
L2 + T2	78.9	98.3	100	100	–	–	–	70.8	99.0	100	100	–	–	–
L2 + T3	89.8	99.7	100	100	–	–	–	89.0	98.6	100	100	–	–	–
L2 + T4	46.1	73.4	76.4	79.5	78.6	75.4	97.0	78.2	87.9	91.2	93.7	99.8	100	–
L2 + T5	58.8	79.2	90.4	98.5	96.4	96.4	100	54.8	77.9	86.9	87.0	95.2	97.8	99.9
L2 + T6	55.9	85.2	78.0	85.3	88.6	99.1	100	54.4	93.5	95.8	97.4	100	–	–
L2 + T7	40.7	75.6	72.2	80.5	88.6	98.2	97.6	48.2	43.0	50.8	36.6	39.6	45.9	50.6
L2 + T8	43.3	30.9	40.4	55.2	42.6	47.9	63.4	60.6	59.2	57.1	52.7	40.9	49.6	42.2
L2 + T9	94.1	88.2	86.0	91.1	98.2	99.5	100	85.2	99.0	99.1	100	100	–	–
L8 + T1	38.6	33.8	31.8	29.3	15.9	6.1	3.6	38.8	41.0	32.3	19.4	21.9	12.3	19.2
L8 + T2	79.5	84.9	100	100	–	–	–	77.2	97.0	100	100	–	–	–
L8 + T3	86.2	98.9	70.8	59.2	49.6	35.9	22.7	77.2	98.3	100	100	–	–	–
L8 + T4	57.8	90.1	73.5	77.9	74.3	72.2	59.3	61.0	60.2	68.7	51.5	40.6	31.2	13.0
L8 + T5	60.5	76.8	89.5	86.5	76.1	82.4	99.2	56.6	86.3	94.0	100	–	–	–
L8 + T6	64.7	70.0	89.4	95.8	89.6	96.6	100	42.8	62.6	79.6	82.1	83.4	95.7	98.6
L8 + T7	64.7	62.1	68.7	42.3	39.3	35.3	44.8	27.6	29.9	38.4	31.7	34.1	9.6	9.3
L8 + T8	46.5	29.0	43.5	46.2	33.5	30.0	54.7	40.4	61.1	54.6	44.8	49.6	54.0	58.6
L8 + T9	85.5	90.2	89.6	95.5	99.9	100	–	92.0	99.1	100	100	–	–	–

peated passages through the host plant. These were L-2 + T-8 and L-8 + T-8 at both Aberdeen and Bozeman, L-8 + T-4, L-8 + T-7, at Aberdeen only, and L-2 + T-7 at Bozeman only.

For the most part there was close agreement in the results obtained at Aberdeen and Bozeman. One notable exception, however, was the behavior of the L-2 + T-7 mixture. After 7 passages through the host 97.6 per cent of the chlamydospores of this mixture were the L-2 type at Aberdeen, while the 2 species were present in approximately equal proportions at Bozeman. Similar results were obtained with mixtures incorporating L-8 with T-4 and T-7. These mixtures were highly compatible at Aberdeen, while at Bozeman T-4 and T-7 greatly predominated after the seventh passage through the host. These differential survivals at the 2 locations could have been caused either by differences in environment or by modification of the original mixture through hybridization.

In addition to experiments with species mixtures, tests were made with a race mixture involving L-2 and L-8. Since the chlamydospores of these races are identical in morphology, pathogenicity tests were made to determine which race survived 5 passages through Ulka wheat at Aberdeen and at Bozeman. Inoculum from the fifth passage through Ulka at both locations was used to inoculate seed of the standard differential varieties (7). The winter wheat varieties were grown at Pullman, Wash., and the spring wheats at Aberdeen, Idaho. It was evident from the results that the surviving spores of the original mixture were those of L-8. Apparently, therefore, L-8 greatly suppressed, and possibly eliminated, L-2 from the mixture.

The nature of the differential survival ability indicated above is not known. The possibility that 1 species exerts an antagonistic effect on the other was explored by growing the various combinations together on an artificial medium. This was done by placing the primary sporidia from a single promycelium of 1 species on a hanging drop of 4 per cent potato-dextrose agar near those from the promycelium of the other species. The sporidia were separated from each other sufficiently to prevent immediate fusion. Isolation and placement of the sporidia was done with the aid of a micromanipulator. Four replications of each combination shown in Table 1 were made and observations were made daily for 1 week. In every case the mycelia of the 2 sporidial sets grew together indiscriminately, intermingled freely, and finally coalesced into 1 colony. Thus, there was no indication of detrimental or antagonistic effects of the mycelial development of 1 race toward another. In some cases the mycelial growth of L-2 and L-8 was slower than that of other races.

Rate of spore germination as a basis for differential survival was also investigated. Replicated germination tests on all races were made by planting chlamydospores on 4 per cent water agar and incubating them at temperatures ranging from 10°–20° C. Only 1 consistent difference in rate of germination between the "T" and "L" races was observed. Spores of L-2 and L-8 consistently germinated about 48 hours earlier than T-9. Thus, the high percentage of recovery of these 2 races from mixtures with T-9 in the first passage through the host (Table 1) possibly could be due to the relatively slow rate of spore germination in T-9.

HYBRIDIZATION.—Hybridization between *Tilletia caries* and *T. foetida* occurs readily under controlled conditions and the hybrid spores are less prominently reticulate than those of the reticulate-spore parent (1, 4, 6). Furthermore, there is evidence that hybridization between these species occurs under natural condition (2, 3). The use of species mixtures of spores in studies on competition affords ample opportunity for inter-specific hybridization through infection by the product of fused primary and secondary sporidia. That this occurred in at least 1 of the spore mixtures

TABLE 2.—*The relative prevalence of 3 kinds of chlamydospores in the mixture L-8 + T-3 after each of 7 passages through the host at Aberdeen, Idaho*

Original mixture	Spore types	Percentage of each type of spores present at indicated passages through the host						
		First	Second	Third	Fourth	Fifth	Sixth	Seventh
L-8 + T-3	L-8	86.2	98.9	70.8	59.3	49.5	35.2	22.7
	T-3	13.8	0.9	7.2	6.0	8.0	9.5	16.3
	hybrid	0.0	0.2	22.0	34.7	43.5	55.3	61.0

(L-8 + T-3) used in the competition study was indicated by the occurrence of hybrid-like spores (5) in this mixture after the second passage through Ulka wheat. A study was undertaken with this material to determine whether additional evidence that hybridization had occurred could be obtained. The results are summarized in Table 2.

The data in Table 2 show that, in the mixture of L-8 + T-3, 86.2 per cent of the spores were L-8 after the first passage through Ulka at Aberdeen. The remainder were typical T-3 spores. After the second passage through Ulka, the percentage of L-8 increased to 98.9, that of T-3 decreased to 0.9, and 0.2 per cent were so minutely reticulate as to indicate they were of hybrid origin (5). With the third passage through Ulka these percentages changed to 70.8 for L-8, 7.2 for T-3, and 22 for the hybrid-like spores. In succeeding passages through the host, L-8 continued to decrease while the reticulate spores increased to a predominant proportion after the sixth passage through the host. At the seventh passage the percentages were 22.7, 16.3, and 61 for L-8, T-3, and the hybrid, respectively. Apparently, therefore, the hybrid spores were more aggressive than those of either parent. On the other hand, they may have had a lesser depressing influence on T-3 than did L-8. This is suggested by the fact that, although decreased to 0.9 per cent after the second passage, (in competition with L-8 only) T-3 increased to 16.3 per cent at the seventh passage. At the same time L-8 decreased to 22.7 per cent. Obviously, however, this resurgence of T-3 could have stemmed from segregation and recombination of factors governing spore markings.

As noted earlier the same mixture (L-8 + T-3) produced entirely different results in the tests at Bozeman (Table 1). Under these conditions T-3 was eliminated with the third passage through Ulka and no hybrid-like spores were in evidence. This further supports the suggestion that hybridization accounts for the discrepancy in results with this mixture at the 2 locations. Pathogenicity tests were not made because lots of the hybrid spores that were free from admixtures of L-8 and T-3 could not be isolated.

SUMMARY AND CONCLUSIONS

It seems evident from this study that species and races of the bunt fungi differ in their respective abilities to survive passage through a susceptible host in mixed populations. In general the "L" races exhibited more aggressiveness than the "T" races. In some mixtures spores of the "L" races predominated after the first passage through the host. In others, 2 or more passages were required for predominance of the "L" race spores. Races that were exceptions to the above rule were T-1, T-4, and T-7. Of these 3 races, T-1 was the most aggressive in competition with the "L" races.

No explanation can be offered for the demonstrated differential ability of species and races of *T. caries* and *T. foetida* to survive passage through the host in combination with each other. Rate of spore germination may be at least a contributing factor in some cases. Antagonistic effects on mycelial growth apparently have no influence in this respect.

There is ample opportunity for natural hybridization to occur in mixed populations of spores representing different species or their respective races. A hybrid thus produced may or may not give rise to lines of more aggressive tendencies than the parent stock. In this study 1 mixture (L-8 + T-3) gave rise to hybrid spores which predominated in the spore population after 7 passages through the host.

Information gained from this investigation may help to explain the relative prevalence of species and races of *T. caries* and *T. foetida* on regional and local levels.

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LEAFHOPPER-VIRUS RELATIONSHIPS OF PEACH YELLOW LEAF ROLL[1]

D. D. Jensen

Yellow leaf roll disease of peach, occurring primarily in the main peach growing area of northern California, was shown to be due to a graft transmissible virus by Nyland and Schlocker (4). In 1952, Jensen et al (3) proved experimentally that the geminate leafhopper, *Colladonus geminatus* (Van Duzee), transmitted yellow leaf roll virus from peach to peach in the greenhouse. The minimum demonstrated latent period of the virus in the leafhopper vector was found to be approximately 30 days and in most groups tested it was over 40 days. Since these tests involved groups rather than single insects and as the acquisition feeding time was at least 5 days and the test feeding time at least 7 days, neither the exact latent period nor the number of individuals becoming viruliferous could be determined precisely.

This report (3) also reviewed the symptom similarity of yellow leaf roll and western X-disease and revealed that the 2 diseases have 1 or more vectors in common, and an equally long latent period of the virus in the vector. The insect vector relationships thus provide additional evidence that the casual viruses are related and may be strains in the same virus complex, because according to the facts now available, it is uncommon for a species of leafhopper to transmit unrelated viruses. Moreover, the latent period of the 2 viruses in the insect vector is apparently of the same abnormal length.

The present paper reports experiments involving variable acquisition feeding periods and provides incidental information on the latent period of the virus in the vector and on the pattern of virus transmission by individual insects.

MATERIALS AND METHODS.—The test insects (*Colladonus geminatus*) were reared in the greenhouse on celery. They were transferred to the inoculum trees during the nymphal stage for the acquisition feeding period and then returned to celery where all became adults before being tested on healthy peach.

The yellow leaf roll plants used as the virus source were Lovell peach seedlings grown in the greenhouse and infected by means of insect vectors during the 1951 season when the trees were approximately 6 months old. All test trees were Lovell peach seedlings germinated in the greenhouse.

At the time of exposure to the test insects, the trees were growing in 4-in. pots and varied in age from 2 to 7 months. Despite the small size of the pots, foliage growth was successfully maintained throughout most of the test period by occasional applications of cotton seed meal fertilizer and weekly applications of ammonium sulphate dissolved in water. The test trees were cut back from time to time to a height of 4-6 in. so that during the test feeding period they could be enclosed within cages 7½ in. high.

EXPERIMENTAL PROCEDURE.—In an experiment designed to measure the latent period of the virus in the vector more exactly and also to determine the number of individuals becoming infective, nymphs were allowed to feed on a diseased peach tree 2 days and then held on celery for 3 weeks when all had become adults. They were then divided into 2 groups. Group A consisted of 15 males and 15 females which were tested individually for infectivity by allowing each to feed for a period of time exceeding 30 days on single, healthy peach seedlings. In Group B were 5 males and 5 females which were each transferred daily to a new peach tree until they were all killed on the test trees used for the 26th daily transfer because these trees had been sprayed accidentally with tetraethyl pyrophosphate only a few hours before the leafhoppers were caged on them. These 10 leafhoppers were replaced in the daily transfer tests by 5 males and 5 females taken from Group A which, during the intervening time, had been held continuously on single, healthy peach trees. These 10 insects were subsequently transferred to new trees daily until they died. Three additional leafhoppers were tested on peach after having been held on celery for a longer time than those mentioned above. In these experiments, only 2 of the 43 leafhoppers tested proved to be viruliferous, and only 4 trees developed disease symptoms out of 809 trees exposed to feeding by the 43 test insects. The meager results could have been due to the use of a poor virus source, an inadequate acquisition feeding period, or both.

Acquisition feeding period.—The effect of the length of the acquisition feeding period on the number of insects becoming viruliferous was tested by varying the time the insects were given access to a constant virus source.

The acquisition feeding periods tested were 1, 5 and 10 days. After the insects were removed from the inoculum plant, they were held on celery for 37–42 days when 20 males and 20 females from each group were transferred singly to separate peach trees. They were left on the first peach trees for 7 days and were then transferred successively to new trees every 7 days until death. When a leafhopper died it was replaced by one from the same stock being held on celery and bearing a separate number.

From the number of individuals of each sex becoming infective at the various acquisition feeding times tested (Table 1) it is evident that a 24-hour acquisition feeding period is insufficient for many leafhoppers to become viruliferous. Only 1 of 64 leafhoppers feeding for this period became infective. With a 5-day acquisition period, 17 of 61 became infective and with a 10-day acquisition period, 9 of 65 became

[1] Accepted for publication June 24, 1953.
The author is indebted to Mr. Ernest J. Ranft for valuable assistance during the work.

TABLE 1.—*Effect of acquisition feeding period on transmission of peach yellow leaf roll virus by Colladonus geminatus (Van Duzee)*

Sex	Acquisition feeding periods		
	1 Day	5 Days	10 Days
	No. infective insects	No. infective insects	No. infective insects
Males	1/37[a]	3/32	7/35
Females	0/27	14/29	2/30
Totals	1/64	17/61	9/65

[a] Numerator is number of leafhoppers transmitting virus and the denominator is the number of leafhoppers tested.

infective. The lower number of viruliferous insects at 10 days than at 5 days appears to be anomalous. The source plant used for all 3 feeding periods was the same. The insects feeding for 1 day and for 5 days were from the same insect colony. However, the insects used in the 10-day feeding period had to be taken from a separate stock colony and this may be a possible explanation for the smaller number that became infective. The fact that 14 females and only 3 males became infective at 5 days is of interest. However, this relation to sex seemed to be reversed in those feeding for 10 days.

Intermittent transmission.—After yellow leaf roll virus has completed the latent period in the insect, transmission to subsequent test trees appears to be irregular and intermittent rather than consistent. In the first experiment discussed, involving daily transfer of single leafhoppers to new peach trees, only 2 individuals proved viruliferous. One of these infected a single tree and failed to infect any of the following 5 it fed upon before it was killed. The other individual infected 1 tree, failed to infect the tree fed upon the following day, infected the next tree, then failed to infect any of the following 19 trees before accomplishing its third infection. It then fed on 19 more trees before death without infecting any of them.

Although the cause is not understood, intermittent transmission of plant viruses by insect vectors is common and has been reported frequently. In fact, failure of single insects to infect every plant fed upon when transferred successively to a series of healthy plants is the expected result. Relatively few individuals infect all the plants they feed on if the test plant series is long. This is true for both persistent and non-persistent insect transmitted viruses.

In the experiment involving acquisition feeding periods of 1, 5, or 10 days, each individual was caged for 7 days on each test plant. Of the 27 leafhoppers which proved to be viruliferous (Table 2), only 2 missed a full week between infections. However, after infecting 1 or more trees, 7 individuals failed to infect only the last plant fed upon, 2 failed to infect the last 2 plants fed upon, and 2 failed to infect the last 3 fed upon. The relatively large number of failures on the last plant in each series is probably due, in part, to the fact that these were the plants on which the insects died and the extent to which these plants were fed upon is unknown. The insects may have died on any of the 7 days during which the plants were caged but not examined.

Latent period.—The latent period of the virus in the 2 infective leafhoppers transferred daily was measurable with close accuracy. In 1 individual it was between 42 and 44 days. In the other individual it was between 59 and 61 days. In the experiment (Table 2) involving weekly transfer of the leafhoppers, after 1, 5, or 10 days on inoculum, the latent period was not determined within close limits, but some groupings can be made which indicate the relative numbers of individuals which fall within the respective periods.

The latent period was between 48 and 55 days in the single individual acquiring virus in 1 day. Among

TABLE 2.—*Transmission pattern of yellow leaf roll virus of peach by single infective Colladonus geminatus leafhoppers after acquisition feeding periods of 1, 5 or 10 days on diseased peach, followed by 37-42 days on celery. Insects were transferred serially to new peach test trees every 7 days*

Acquisition feeding period in days	Leafhopper sex	Successive test trees				
		1	2	3	4	5
1	♂	−[a]	+	−	−	−[b]
5	♂	−	+			
	♂	+	−			
	♂	−	+	−		
	♀	+				
	♀	+				
	♀	+	−			
	♀	+	−			
	♀	+	−	−		
	♀	+	−	−		
	♀	−	−	+		
	♀	+	−	+		
	♀	−	+	+		
	♀	+	−	−	−	
	♀	+	+	+	+	
	♀	+	−	+	+	+
	♀	[c]	+	+	+	−
	♀			−	+	
10	♂	+				
	♂	+				
	♂	+				
	♂	+	−			
	♂	−	+			
	♂	−	+	−		
	♂		−	−	+	−
	♀	+				
	♀		+	+	+	

[a] The minus sign (−) indicates no symptoms produced, and the plus sign (+) indicates production of disease symptoms in the test plant.

[b] The insects died on the last plant of each series for which a plus or minus sign is given.

[c] Blank spaces at the beginning of a series indicate that the respective insects had not yet been started in a test series until the week represented by the first plus or minus sign.

those feeding 5 days on inoculum, the latent period was less than 45 days in 11 insects, between 45 and 52 days in 3, and between 59 and 66 in 1. Among those feeding 10 days on inoculum, the latent period was 52 days or less in 5 insects, 57–64 days in 2, and 64–71 in 1.

Incubation period in the plant.—The time between infection and the appearance of earliest symptoms in peach varied considerably, presumably because of differences in condition or growth rate of the trees. Since each tree was exposed to the vector for 7 days, the actual day of infection cannot be determined. The figures below represent the time between the first day of the vector on the test tree and the date symptoms were recognized. The incubation period in 38 plants ranged from 21 to 90 days. The distribution of cases between these extremes follows: 21-30 days, 4 trees; 31-40, 16; 41-50, 7; 51-60, 7; 61-70, 1; 71-80, 1; 81-90, 2. The mean was 43 days, but the modal period was 31-40 days.

The earliest symptoms on Lovell seedlings, growing in 4-in. pots in the greenhouse, consisted of rolling of the leaf margins upward and inward. This usually occurred on 1 or more of the expanded sub-apical leaves and typically preceded the appearance of a lighter green color in the leaves. The color change appeared first along the veinlets as the rolling became more pronounced. In some instances the progress of the symptoms was very rapid in individual leaves, developing from suspicious to pronounced in 1 week.

Discussion.—The relationship between feeding time and virus acquisition, and in particular the minimum feeding time necessary for insects to acquire virus from diseased plants has been determined for relatively few viruses. This is especially true for those transmitted by non-aphid vectors.

Storey (5) reported what is probably the shortest feeding period known for successful virus acquisition from a plant by a leafhopper. He found that *Cicadulina mbila* could obtain corn streak virus from the plant in a single feeding lasting only 15 seconds. Moreover, a surprisingly high percentage of individuals became infective after short feedings, 11 of 25 becoming viruliferous at 15 seconds and 26 of 53 at 1 minute. Feeding for 5 minutes or more was required for infecting a healthy plant.

Bennett and Wallace (1) found that the beet leafhopper, in a low percentage of cases, could pick up the curly top virus in 1 minute and could infect healthy plants in a similarly short time. They found further that the percentage of leafhoppers becoming viruliferous increased with increased acquisition feeding time up to 5 hours.

It is probable that the brief acquisition feeding times demonstrated for corn streak virus and curly top virus are much shorter than is typical for leafhopper-transmitted viruses in general. It is of interest that these 2 viruses also have relatively short latent periods in their vectors, being measurable in hours rather than in days.

The minimum feeding time necessary for leafhoppers to acquire a stone fruit virus has not been precisely demonstrated. The main reason for this, in the few stone fruit viruses studied, is possibly that when the acquisition feeding period is reduced even to 1 or 2 days, the percentage of individuals becoming viruliferous drops to a very low point. The reason for so few individuals becoming infective in 24 or 48 hours is unknown. It is probable that the leafhoppers can acquire an infective charge of virus during the feeding which follows a single puncture. It is also probable that at greenhouse temperatures the leafhopper makes several feeding punctures every day, so that infectivity is not dependent upon a single feeding puncture being maintained for a prolonged period. A more likely explanation is that the virus may not be consistently available in all the tissues fed on by the leafhoppers; thus the longer an individual remains on a diseased tree, the more times it feeds and the greater becomes the chance that the right conditions for virus acquisition will be encountered.

Hartzell (2) fed *Macropsis trimaculata* for periods of 1, 4, 7, or 10 days on trees carrying peach yellows virus. Transmission was obtained with 7 of 21 groups feeding for 4 days. No transmission occurred in the groups fed for 1, 7, or 10 days. This may have been due, in part at least, to the fact that only a few tests were conducted at each of the negative feeding periods.

In the present work with yellow leaf roll virus of peach, only 1 of 64 geminate leafhoppers acquired virus during a 1 day feeding period, 2 of 43 at 2 days, 17 of 61 at 5 days and 9 of 65 at 10 days. These results suggest that the minimum feeding periods necessary for virus acquisition and transmission can best be determined from experiments using large groups of leafhoppers rather than from insects tested individually.

SUMMARY

When tested individually in daily transfers to 809 peach trees, only 2 of 43 *Colladonus geminatus* (Van Duzee) leafhoppers acquired yellow leaf roll virus of peach during a 2-day acquisition feeding period. Transmission was accomplished to only 4 trees.

In an experiment in which a common source plant was used, but the time the insects were exposed to the inoculum was varied, 1 of 64 leafhoppers became viruliferous during 24 hours, 17 of 61 during 5 days and 9 of 65 during 10 days on the diseased plant.

In successive transfers to healthy test plants at daily or weekly intervals, viruliferous individuals went as long as 19 days between transmissions, but retained the virus up to 72 days.

The latent period of the virus in the leafhopper was found to be as long as 64-71 days although it was below 45 days in most individuals.

The incubation period for symptoms of yellow leaf roll virus in Lovell peach seedlings grown in the

greenhouse ranged from 21 to 90 days with the modal period between 31 and 40 days.

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EFFECTS OF TOBACCO-ETCH VIRUS ON PEPPERS (CAPSICUM SP.) [1]

W. H. Greenleaf [2]

An unknown wilt disease appeared on the Tabasco pepper, *Capsicum frutescens* L., in the breeding plot at Experiment, Georgia, in July 1945. The plants were growing vigorously and had just begun to bloom and to set a few fruits when several wilted as if from drouth. Within about 2 weeks all had wilted and were either dead or dying.

In late summer 1950 a similar wilt disease appeared at Auburn, Alabama in a field of mature Tabasco pepper plants and crosses with Tabasco pepper. This paper describes the identification of the causal agent of this disease and its effect on the Tabasco pepper, on certain other peppers, and on several other plant species. An abstract of the results has been published (2).

MATERIALS AND METHODS.—Indicator and differentiator hosts for tobacco-mosaic virus (TMV) and for tobacco-etch virus (TEV) were used. These were *Nicotiana tabacum* L. var. Holmes' Samsun[3] possessing the localization factor N for TMV from *N. glutinosa* (4, 13), var. Trelease Turkish, var. Turkish Samsun and var. *purpurea*, *N. glutinosa* L., *Datura stramonium* L. (Jimson weed), Tabasco pepper, Holmes' TMV resistant bell peppers possessing the localization factor L for TMV from the Tabasco pepper (3) and F_1 hybrids of the latter with certain inbred lines of the Pimiento pepper. Cucumber was included to detect the possible presence of cucumber-mosaic virus. The following species were tested in a search for a local lesion host for TEV: *Physalis ixocarpa* Brot. (Tomatillo), *P. floridana* Rydb.[4], *Solanum integrifolium* Poir. (Chinese Scarlet Eggplant), *S. carolinense* L. (Horsenettle), *S. pseudo-capsicum* L. (Jerusalem Cherry), *Chenopodium* sp. and *Gomphrena globosa* L. (Globe Amaranth). Representatives of *Capsicum annuum* L., *C. pendulum* Willd., *C. microcarpon* Cav. and probably also of *C. frutescens* L. (12) were tested for resistance to TEV and to TMV.

Extracts of TMV and of TEV were prepared from leaves or shoots that had been crushed in a mortar and strained through cloth. The source of TMV was diseased tomato plants growing in the greenhouse during the winter. This was the common green-mottling strain. It was always tested for contaminants by inoculation into 1 or more known local lesion hosts for this virus, e.g. *N. glutinosa* L., Holmes' Samsun tobacco, Tabasco pepper or Jimson weed. This virus was readily maintained on hosts in which it is systemic, e.g. on Turkish tobacco or on pepper plants. Whole or diluted extracts either freshly prepared or after storage near 32°F. served as the inoculum.

The source of TEV was, except when specifically stated otherwise, a wilted Tabasco pepper plant from the field. TEV was most conveniently maintained on Holmes' Samsun tobacco, thus avoiding contamination with TMV. Only freshly prepared whole extracts were used in inoculations with this virus.

The carborundum leaf-wiping method of inoculation was used in all experiments. The use of carborundum proved essential for the consistent transmission of TEV. This confirms the results of Costa (1) and others. From 1 to 5 strokes with a saturated cloth pad were made on a leaf and the inoculum in the pad was renewed frequently. The leaves were rinsed after a set of inoculations was completed. The hands were washed with soap and water between sets of inoculations, and all feasible precautions were taken to avoid contamination. Inoculations were made

[1] Accepted for publication June 30, 1953.
The writer wishes to acknowledge the contributions of the following fellow-workers to the solution of this problem: Dr. E. S. Luttrell, Pathologist, Georgia Agricultural Experiment Station, Experiment, and Dr. J. L. Weimer (retired), formerly Pathologist, U.S.D.A., at Experiment; Dr. S. G. Younkin, Pathologist, Campbell Soup Co., Riverton, New Jersey; Dr. R. L. Self, formerly Pathologist, Alabama Agricultural Experiment Station, Auburn, now Pathologist, Ornamental Horticultural Substation, Spring Hill, Alabama; Dr. F. O. Holmes, Virologist, The Rockefeller Institute for Medical Research, New York, and Mr. V. G. Perry, former graduate student at Auburn, now Junior Nematologist, U.S.D.A., Sanford, Florida.

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[3] Thanks are expressed to Dr. Roy E. Clausen, Division of Genetics, University of California, Berkeley, for providing seeds of several tobaccos.

[4] Receipt of seeds of *Physalis floridana* and *Gomphrena globosa* sent by Dr. A. Frank Ross, Department of Plant Pathology, Cornell University, Ithaca, N. Y. is gratefully acknowledged.

in a screened greenhouse. Insects were controlled by periodic spraying with parathion. Potted plants were grown in sterilized soil. Inoculations were generally made at temperatures favorable for plant growth, i.e. between 70 and 95°F. Controls were either inoculated with distilled water, tap water, or with extract from healthy plants.

IDENTIFICATION OF THE WILT DISEASE ON THE TABASCO PEPPER.—Luttrell had made an attempt to isolate the causal organism from dying Tabasco pepper plants in Georgia in 1945, but without success. Weimer's opinion that the symptoms were typical of a virus disease had focused attention on this possibility. In 1946 2 plantings of Tabasco pepper were made some distance apart in the same field where the disease had previously occurred. Early in the season 2 plants were enclosed in individual insect-proof cloth cages. The disease reappeared and killed all except the 2 caged plants. Only Tabasco peppers in the collection were thus affected.

In late summer, 1949, Perry and the author had been successful in transmitting the disease from a wilted Tabasco pepper plant from the field of 2 of 4 healthy potted plants of Tabasco pepper when the leaves were rubbed with extract from the diseased plant without the use of carborundum (Fig. 1). In late summer 1950 the wilt disease occurred in a much larger planting of Tabasco pepper and of crosses with Tabasco pepper at Auburn, Alabama (Table 1).

The author considered TMV or TEV as possible causal agents of the disease. To distinguish between TMV and other viruses 12 plants of Holmes' Bell peppers homozygous for the L gene were inoculated with extract from a wilting Tabasco pepper plant. All 12 developed a systemic mottle, indicating that the virus in question was not TMV. When 14 Tabasco pepper seedlings were inoculated with extract from these plants, all developed the wilt disease. Three samples of peppers of the L genotype including Tabasco that had been inoculated as described were sent to Dr. Holmes for identification. He concluded that the virus was a strain of severe etch. Inoculations by the author with the above mentioned virus isolate into Holmes' Samsun tobacco, Turkish tobacco, and Jimson weed produced the symptoms that are diagnostic for TEV (7), thus confirming Dr. Holmes' finding. In the 2 tobaccos primary yellow spots developed which soon became surrounded with single or double etched rings. A general etching over the inoculated leaf followed. Secondary symptoms were yellow spots, veinclearing and veinetching that appeared first in the lower portions of the leaves above the inoculated leaf. Etching later appeared in the other leaves. In the shoot leaves these symptoms began at the tips and spread toward the base of the leaves. Etching of the type described is produced only by severe strains of etch. On Jimson weed no primary symptoms were produced. Secondary effects were veinclearing in the young shoot leaves followed by the development of severely distorted, filiform, and mottled leaves. Cucumber plants remained symptomless. Numerous subsequent transfers of this virus isolate to several of the other hosts mentioned above revealed no evidence to doubt that this virus was a strain of severe-etch virus *Marmor erodens* var. *severum* H.

FIG. 1. Tabasco pepper plant about 14 days after inoculation with extract from shoots of a wilted Tabasco pepper plant from the field. No carborundum was used. Note the recovery within 3 hours of a shoot from a similarly wilted plant when severed and set in water.

RESPONSE OF TABASCO PEPPER TO TEV.—The first visible symptoms following infection of Tabasco pepper with TEV were veinclearing and faint yellow flecks in the young shoot leaves. These symptoms appeared in from 4 to 21 days after inoculation and were invariably accompanied or followed by wilting within a few days. The time of onset of symptoms varied greatly with the concentration of TEV in the inoculum. With a concentrated inoculum, wilting occurred within

TABLE 1.—*Field observations of the effect of tobacco-etch virus on Tabasco and Tabasco-derivative peppers, Auburn, Alabama, October, 1950*

Population	Classes and number of virus infected plants				
		Susceptible	Tolerant	Resistant	
	Dead	Severely [a] affected	Less affected	Symptomless	Total
Tabasco	92	4	0	0	96
P.I. 152221 (Peru)	0	0	0	8	8
F_1 (P.I. 152221 × Tabasco)	26	14	0	0	40
F_1 (P.I. 152221 × Tabasco) × Tabasco	132	39	1 [b]	1 [b]	173
F_2 (P.I. 152221 × Tabasco)	26	26	9	5	66
F_1 (Tabasco × P.I. 152225)	9	6	0	0	15

[a] Most of these plants were severely defoliated.
[b] No etch-tolerant plants are expected from the backcross to Tabasco. Inoculation of F_1 (P.I. 152225 × Tabasco) has shown that the resistance of P.I. 152225 and by inference that of P.I. 152221 is recessive to the wilt reaction of Tabasco. Hence only the F_2 population is expected to segregate resistant plants.

6–10 days after inoculation in 100 per cent of the plants. Wilting was usually followed by death, but occasionally a few of the young plants about 8 weeks from seed showed partial recovery from the disease. Such seedlings became severely defoliated, but maintained an apical tuft of leaves, eventually producing some new shoot growth and a few fruits on some of the plants. However, observations made over a 1-year period, showed that they remained severely stunted. Both types of response occurred in a single experiment. Larger, older potted plants of Tabasco pepper always died from the disease.

Wilted Tabasco pepper plants showed rapid recovery when their stems were severed and placed in water (Figs. 1, 2). When the stems of wilted seedlings were severed at the soil level, treated with rootone, and placed in a coarse sand rooting medium, they produced only a few roots during a 4-month period (Fig. 2).

RESPONSE OF THE PEPPERS P.I. 152225 AND S.C. NO. 46252 TO INFECTION WITH TEV.—Younkin, in correspondence with the author, reported in 1946 that P.I.

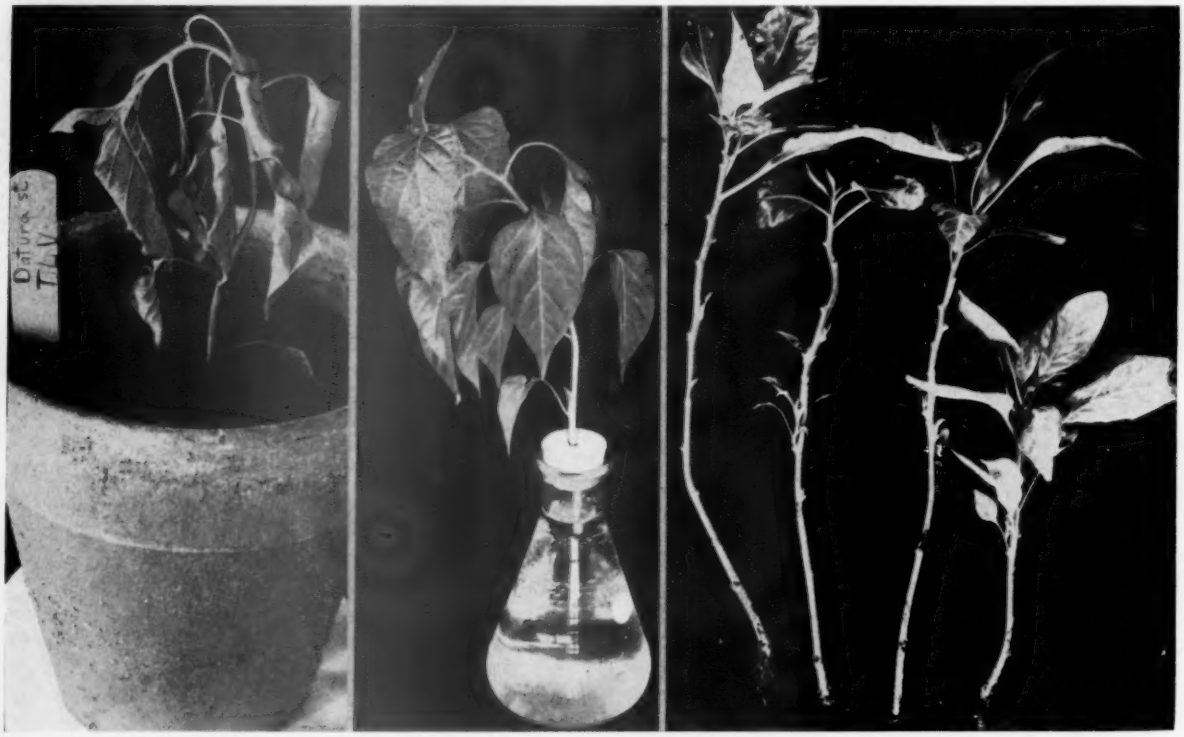

FIG. 2. Effect of tobacco-etch virus on Tabasco pepper seedlings. Left, wilted seedling inoculated on February 15, 1951 and photographed about 15 days later. Center, the same plant 1 day after it had been cut at the soil level and set in water. Right, seedlings from the same lot 4 months later after having been treated with rootone and placed in a sand rooting medium in the greenhouse. Note that only a few roots were produced and that little shoot growth occurred.

TABLE 2.—*Multiplication and translocation of tobacco-etch virus in P.I. 152225 and S.C. 46252 as shown in sub-inoculations to Tabasco pepper plants*

Host	Source	Days after inoculation when sub-inoculations were made		
		2	10	18
P.I. 152225	Inoculated leaves	0/8 (14,34)[a]	8/8 (6)	6/8 (6) 8/8 (11)
	Noninoculated new-growth shoots		0/8 (6,26)	2/8 (6) 6/8 (8) 8/8 (11)
S.C. 46252	Inoculated leaves	whole[b] 0/2 (24) dil. 1/2 (24)	whole 2/4 (14,31)	whole 5/5 (9) dil. 5/5 (9)
	Noninoculated new-growth shoots		whole 0/4 (14,31)	whole 5/5 (9) dil. 5/5 (9)

[a] The numerator of the fractions gives the number of plants that became infected and wilted, the denominator the total number that were inoculated. Numbers in parentheses are days after inoculation. Brackets include plants of the same sample.

[b] This was a 4-day sample. In some tests extracts were used whole and also diluted 1:1 with distilled water.

152225[5] had never shown any signs of infection following inoculation with TEV. The kind or level of resistance, whether immunity or tolerance to TEV, possessed by this introduction, was of practical importance to pepper breeders. Since visible symptoms of infection with TEV were either absent or indistinct, the following experiment was conducted. About 4 marked (punctured) leaves were inoculated on each of 24 young, rapidly growing plants. The plants were inoculated in groups of 8, each with fresh inoculum of a high titer 7-day source of TEV from Holmes' Samsun tobacco. Samples were taken at intervals of 2, 10, and 18 days after inoculation from the marked, inoculated leaves and after 10 and 18 days from whole noninoculated tips of new-growth shoots. Extracts from each were tested on Tabasco pepper plants. Results are in Table 2. Inoculated leaves did not contain sufficient virus after 2 days to be assayed on Tabasco pepper seedlings, but they did after 10 days. Virus was not yet present, however, in detectable concentration in the shoots at this time. After 18 days the virus was also present in the shoots, accompanied by a faint mottle in some, but not in all the plants. Twenty of 24 plants were visually diagnosed positive 41 days after inoculation. The 4 remaining symptomless plants were thought to have been those from which the inoculated leaves were removed for the 2-day sample assay before the virus had passed into the plant. The most characteristic and dependable symptom was veinbanding of the top shoot leaves which showed as a faint, at times barely perceptible mottle.

McKinney (9) reported an etch-immune pungent pepper, South Carolina No. 46252[6]. The author tested it in a manner similar to that already described for P.I. 152225 except that a second isolate of TEV indistinguishable from the first and obtained from a mosaic-diseased Pimiento plant from the field was used for these tests. Successive transfers through tobacco var. *purpurea*, Holmes' Samsun tobacco, Tabasco pepper, Holmes' Samsun tobacco and *N. glutinosa* had freed this isolate from an associated virus diagnosed to be a strain of TMV.

Twenty-eight plants of S.C. No. 46252 growing rapidly in a shaded greenhouse were each inoculated on 4 marked (punctured) leaves on August 26 with extract from *N. glutinosa* plants 13 days from inoculation. Five Tabasco pepper plants inoculated at the end and wilted within 6 days after inoculation demonstrated the virulence of the inoculum. Sample extracts were made from inoculated leaves only from 6 plants after 4 days and from both inoculated leaves and shoot tips from 6 or 10 plants 10 and 18 days after inoculation and tested on Tabasco pepper plants. Results were similar to those obtained with P.I. 152225 concerning the delayed translocation of the virus from the inoculated leaves to the shoot (Table 2). Only a limited amount of virus could be recovered from the 10-day sample of inoculated leaves and none from the shoots but after 18 days both were positive. None of the plants showed symptoms at the time the extracts were made. Sometime between 18 and 31 days, however, 16 of the 28 plants had developed a distinct veinbanding and mottle in the shoot leaves indicating susceptibility to the virus. Young axillary shoots showed mottled leaves with downcurved tips. Similar slight distortion had been noted on young axillary shoots of P.I. 152225. The other 12 plants were recorded symptomless at this time. The temperature was favorable during this period, ranging from about 70 to 95°F., and the plants grew rapidly.

Two tests were made of extracts from symptomless plants. In the first test extract was taken from 4

[5] P.I. 152225 and P.I. 152233 of Greenleaf, and P.I. 159241 of Younkin proved to be identical. Younkin's line was from seed harvested by the author in 1945 and sent for redistribution to the U.S.D.A. Division of Plant Exploration and Introduction, Washington, D. C.

[6] A Cayenne pepper from J. A. Martin, Department of Horticulture, Clemson College, Clemson, South Carolina.

shoots of 4 plants 30 days after inoculation. Inoculum consisted of 3 cc. of whole extract diluted with 5 cc. of distilled water. Five inoculated Tabasco pepper plants and 5 tap water checks were negative. In the second test 2 extracts were made 42 days after inoculation, one from 20 inoculated leaves and the other from the shoots of 5 plants, including the 4 that already had been tested and found negative. Both extracts were tested undiluted on 10 and 5 Tabasco pepper plants, respectively. All were negative.

In a third test, also made 42 days after inoculation, an extract was made from the main shoot and from 1 basal shoot of a plant that was a carrier suspect. It had diffuse yellow spots in some leaves of both shoots and also some faint veinbanding in certain of the young leaves of the basal shoot, but no mottling. Superficially it appeared symptomless and had been so classified previously. Its extract showed positive on 5 Tabasco pepper plants on the eighth day after inoculation. Axillary shoots that developed after removal of the main shoot showed a distinct mottle.

A count made 62 days after inoculation comprised the following symptom categories: 18 + mottled, 2 probably + but showing only well-scattered large diffuse yellow spots in a few of the intermediate leaves, 1 + ? with doubtfully virus-caused veinbanding, 1 + ? with doubtfully virus-caused yellow spots along the periphery of a few of the intermediate leaves and 6 —, e.g. without observable symptoms. Several of the symptomless plants were reinoculated with the first isolate of TEV in extract of Holmes' Samsun tobacco on January 20. Three of these were assayed on Tabasco pepper plants on May 5. Only 1 proved positive, wilting 5 of 8 tester plants. This plant which previously had appeared symptomless developed a distinct mottle after the removal of the shoot tips for assay, but new growth that developed later was again symptomless.

RESPONSE OF OTHER PEPPERS AND OF VARIOUS OTHER SPECIES TO INFECTION WITH TEV.—Two varieties of *Capsicum pendulum* Willd. (11), P.I. 152234[7] with orange colored fruits and P.I. 152235 with red colored fruits, P.I. 152217 and P.I. 152221, from T. B. Barduzzi, Lima, Peru and the commercial variety Serrano were tolerant to infection with TEV. The pepper varieties P.I. 152222 from Barduzzi, P.I. 152453 from W. A. Archer, Para, Brazil, Santanka, Red Chili, Mexican Chili and Burlington were more severely affected. *Capsicum microcarpon* Cav. from A. Piovano, San Juan Province, Argentina, also was severely affected. *Solanum pseudo-capsicum* was tolerant, showing only systemic yellow spots in the leaves. *S. carolinense* was mildly affected, showing veinclearing, mottling, and some puckering of the leaves. *S. integrifolium, Physalis floridana* and 3 strains of *P. ixocarpa* P.I. 15169 from Mexico[8] were severely affected. *Gomphrena globosa* remained symptomless. None of the above hosts produced local lesions with TEV.

RESPONSE OF VARIOUS CAPSICUM SPECIES AND OF SOLANUM PSEUDO-CAPSICUM TO INFECTION WITH TMV.—The localization reaction to TMV was produced in the various *Capsicum* species P.I. 152221, P.I. 152222, P.I. 152225[9], P.I. 152234, P.I. 152235, P.I. 152453, S. C. No. 46252[10], *Capsicum microcarpon* and in *Solanum pseudo-capsicum*[11]. Extensive stem lesions from invasion through the persistent petioles were observed on P.I. 152222, P.I. 152234 and P.I. 152235 but on none of the others. Santanka and the commercial pungent pepper varieties Red Chili, Mexican Chili and Serrano were invaded systemically. Serrano exhibited systemic necrosis.

A NEW LOCAL LESION HOST FOR TEV.—A local weed, tentatively assigned to *Chenopodium album* L. (Lamb's-quarters, Pigweed)[12] but resembling *C. paganum* Reichenb. in the pigmented stems and coarse, tall habit of growth, was inoculated with TEV on the basis of a report that *C. hybridum* was a local lesion host for cucumber-mosaic virus (10). This *Chenopodium* species proved to be a new local lesion host for TEV. With TEV in extract of *N. glutinosa* or in that of Holmes' Samsun tobacco, sharp, distinct lesions developed (Fig. 3 A, B). Three days after inoculation they appeared as diffuse, circular yellow spots, some with a pale center. After 6 days they had developed a necrotic center surrounded with a yellow-green halo. A dark red pigment developed between the necrotic center and the halo in about 7 days. Lesions were best for counting at 9 days. Limited tests showed that this tester plant might be suitable for quantitative work (Fig. 3B). Holmes (5, 6) has reported other local lesion hosts for TEV.

The environment in which plants of the above *Chenopodium* species were grown prior to inoculation greatly affected the quality of the lesions. In early summer young plants growing wild in partial shade were compact in habit and had large, broad leaves that produced clear, sharp lesions which attained a diameter of about 1 mm. in 7–10 days. Plants grown in the greenhouse from seed were tall and open, and had smaller leaves which produced poor lesions. Wild plants inoculated soon after they were transplanted to the greenhouse produced large, sharp lesions (Fig. 3A), but after 2–3 months in this environment their reaction changed. The lesions became poor, and sometimes even failed to develop.

TEV in Pimiento pepper and in Tabasco pepper extracts failed to produce lesions on the *Chenopodium* tester. In mixtures the *Chenopodium* extract completely inhibited the transmission of TEV in tobacco

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[7] The writer's attention was first called to the mosaic resistance of this form of *C. pendulum* in 1950 by A. H. Dempsey, Experiment, Georgia.

[8] Seeds of *P. ixocarpa* were kindly provided by Mr. C. F. Andrus, Southeastern Vegetable Breeding Laboratory, Charleston, South Carolina.

[9, 10, 11] Reported previously by Younkin, McKinney, and Holmes, respectively.

[12] Identification was kindly made by Dr. Earl L. Core, Department of Botany, West Virginia University, Morgantown.

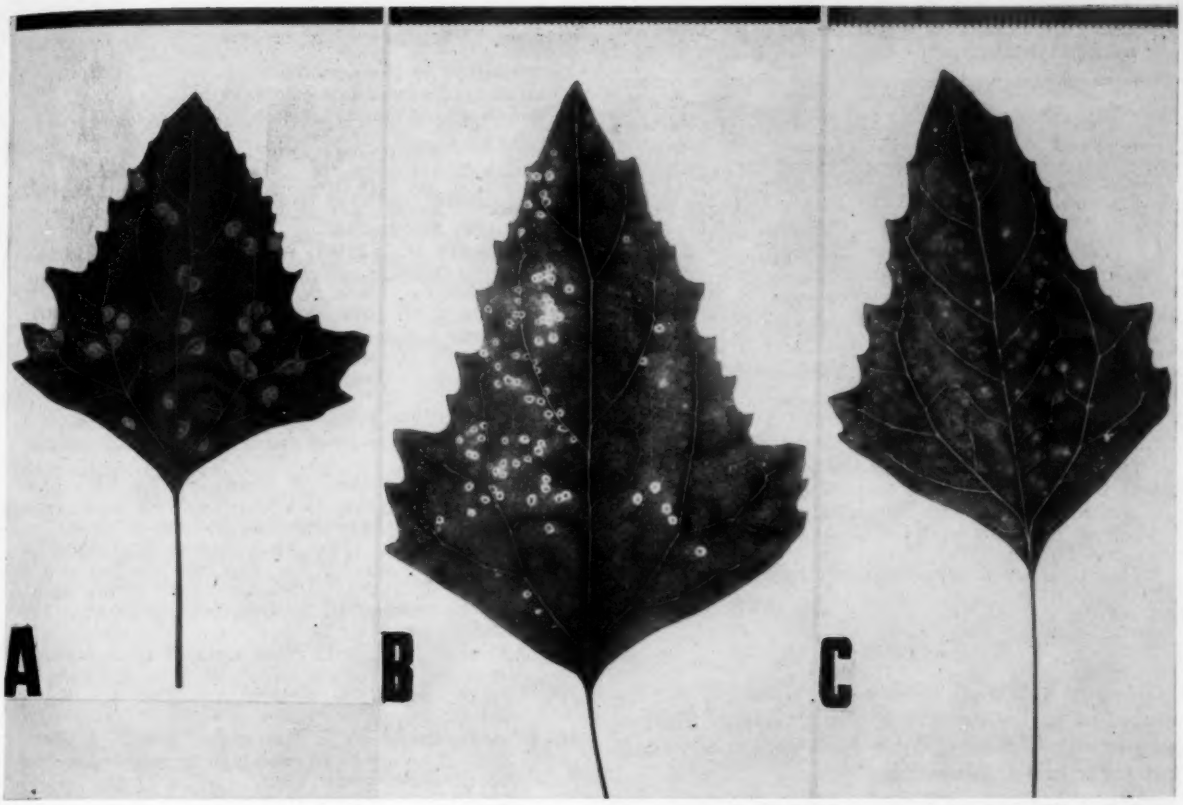

FIG. 3. Response of *Chenopodium album* L. to infection with tobacco-etch virus and with tobacco-mosaic virus. Scale units are mm. A) Leaf from a wild specimen transplanted to the greenhouse and inoculaed about 2 weeks later (June 27) with TEV extract from *N. glutinosa* (47 day source). Photographed 16 days later. Note that the circular lesions develop a necrotic center surrounded by concentric red rings and a yellowish halo. B) Quantitative response to TEV extract from Holmes' Samsun tobacco (7 days source). The left half-leaf was inoculated with a 1/10 dilution, the right half-leaf with a 1/100 dilution of whole extract with distilled water. Photographed 9 days after inoculation. C) Response to TMV extract from Trelease Turkish tobacco. The left half-leaf was inoculated with a 1/2500 dilution, the right half-leaf with a 1/125000 dilution with distilled water. Photographed 6 days after inoculation. The diffuse yellow spots gradually become necrotic.

and in pepper extracts (Table 3). The common occurrence of virus inhibitor substances in the *Chenopodiaceae* has been reported by Kuntz and Walker (8). Both TMV and TEV were inhibited in a TMV-TEV mixture in Pimiento extract (Table 3). When the *Chenopodium* tester was inoculated with TMV in tobacco extract diffuse yellow spots developed on the inoculated leaves in about 6 days. (Fig. 3C). Their diffuseness made them useless for quantitative work. In the greenhouse, leaves that were inoculated with either virus in tobacco extract eventually turned yellow and abscissed. Outdoors, abscission of TEV inoculated leaves occurred in about 20 days. This species of *Chenopodium* appeared immune from systemic invasion by either virus.

DISCUSSION.—Resistance to TEV in the 2 peppers that were tested extensively involves 3 major factors, viz. slow multiplication of the virus in infected tissue, tolerance to infection, and ability to resist infection. The first 2 factors enter into the resistance of P.I. 152225 and all 3 factors enter into that shown by S.C. No. 46252. None of the peppers tested were immune from TEV. However, the levels of resistance that were found in P.I. 152225 and in S.C. No. 46252 offer promise for breeding resistant varieties. The other etch-tolerant peppers require further testing.

Differences in virulence between strains of TEV or differences in environmental factors might explain the conflicting results of McKinney with S.C. No. 46252.

The same gene that determines localization to TMV in the Tabasco pepper apparently is present in several other species of *Capsicum* as well as in *Solanum pseudo-capsicum* since the localization reaction to TMV was found in all of them. Differences in susceptibility to stem invasion were observed. Plants of P.I. 152453, for example, promptly shed the inoculated leaf and developed no stem lesions, whereas plants of P.I. 152234 and P.I. 152235 developed large necrotic areas in the inoculated leaves and had a tendency to develop severe stem lesions through the persistent petioles. L gene peppers also exhibit this undesirable tendency in some degree, but it is probably unimportant under conditions of natural infection and need not necessarily discourage their use in breeding.

TABLE 3.—*Inhibition of TEV in tobacco extract and of a TEV-TMV mixture in pepper extract by C. album extract in* 1:1 *dilutions*

Virus and source plant	Diluent	Tester host	Reaction of inoculated plants
TEV (*N. glutinosa*)	dist. water	Tabasco	2/2[a]
		Samsun	4/4
	Pigweed extract	Tabasco	0/4
		Samsun	0/4
	Pigweed extract + $CaCl_2$[b]	Tabasco	0/8
		Samsun	0/8
TMV + TEV (Pimiento)	dist. water	Tabasco	5/5[c]
	Pigweed extract	Tabasco	0/5
	Pigweed extract + $CaCl_2$[b]	Tabasco	0/5

[a] The numerator of the fractions gives the number of plants that became infected and wilted, the denominator the total number that were inoculated.

[b] 0.75 cc. of 10 per cent $CaCl_2$ was added per 10 cc. of the mixed extracts.

[c] TMV local lesions preceded vein clearing and wilt from TEV.

SUMMARY

A severe strain of tobacco-etch virus (TEV) was proved to be the cause of a wilt disease of Tabasco pepper. The Tabasco pepper was shown to be a sensitive tester host for this virus.

Several peppers belonging to different species were inoculated in a search for sources of resistance for breeding. The pungent peppers P.I. 152225 from Peru and South Carolina No. 46252 were resistant to TEV but not immune. These 2 peppers should prove useful in breeding resistant varieties.

Seven new peppers belonging to several different species produced a localization reaction to TMV.

A new local lesion host for TEV, tentatively assigned to *Chenopodium album* L. (Pigweed, Lamb's-quarters), was studied and reported.

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HEAT CANKER: A PRIMARY PHASE OF COLLAR ROT ON PEANUTS [1]

Lytton W. Boyle

Heat canker has been reported as a primary phase in the development of collar rot, a disease of peanuts. (1). Further data with respect to the development and contingent relationship between these are here presented.

The term "heat canker" is chosen to designate the morbid condition or disease caused by exposure of peanut stems to excessive amounts of radiation at the soil level. This term has been used in both a general (6) and specific sense (5). Here, as in the case of flax (5), the term is limited to a specific type of canker at the soil level on relatively young peanut plants. Heat canker may occur discretely as a nonparasitic disease, or as a primary part in the development of collar rot on peanuts.

The name "collar rot" has already been proposed for a disease of peanuts due to an undetermined cause (2). The description of this disease applies very well to specimens produced by experiment during this study. Collar rot applies to the result when certain etiological factors are integrated in the following chronological manner. A discrete, nonparasitic lesion with more or less morbid tissue intact, such as heat canker, chemical burn, etc., is prerequisite to the development of rot by saprophytic or weakly parasitic organisms.

Occurrence.—In 1949, farmers requested diagnosis of a number of severe cases of heat canker and collar rot on peanuts, traces of which were found in a majority of fields. During 1950, none was found by survey or reported by growers as in 1949. Again in 1951, a number of severe cases was called to our attention by growers and a limited survey indicated that the trouble was prevalent, but on the whole there was less than in 1949. The potentiality of heat canker followed by collar rot has been shown in several fields where it was necessary to reseed. In an extreme case, 75 per cent of the plants were injured in various degrees ranging from lethal to a trace, not evident without magnification.

No statistical estimate has been made of the loss caused by these diseases. As described later, injury is the result of independent and variable factors with respect to meteorological conditions, physical characteristics and conditions of the soil, stage of crop development, etc. An integration of such independent and highly variable factors, sufficient to injure, is not likely to extend beyond the limits of a respective field. For this reason, these diseases show no continuity or gradient in distribution. Under such circumstances, arbitrary estimates are not feasible and statistical estimates would involve data beyond the scope of this study.

Symptoms.—Heat canker is distinguished by its location on the peanut stem with respect to the soil level and exposure to the afternoon sun. At the latitude of southern Georgia, cankers occur on the west to southwest exposure of the stems. Except for this consistency in location the injury may at times be confused with damage by chemicals, insects, or careless cultivation. Cankers vary in severity from superficial microscopic proportions to readily apparent deep lesions. At an early stage, lesions may appear as slight bruises. Later, they may or may not retain a cover of dry, dead tissue. If the injury was originally deep, or if considerable growth occurred after injury, the lesions appeared more or less concave.

Fig. 1. Heat canker from artificial radiation at the primary node; left, control plant; center, open lesion; right, collar rot following injury.

Diseased plants may be roughly classed into 3 types, based on location of the canker or lesion. More than one of the 3 types rarely occur together in a field. The injury is most commonly found at the first node of the plant (Fig. 1). This injury occurs shortly after emergence while the plant has a limited foliage and before soil has been moved about it by cultivation. Unless such injury is severe, it may not be noticed at first. Such plants appear slightly stunted,

[1] Accepted for publication July 2, 1953.

FIG. 2. Heat canker at soil level about older plant. Note small lesions on main stem above third node; burnt tip branch extending to the right from second node.

or may die if collar rot follows the injury. At this time, enough soil may have been moved about the plant by cultivation to make the disease appear as originating beneath, instead of at the soil surface.

A second type of specimen shows injury at the growing tips of the plant. This type of injury may develop after young plants have been covered by cultivation. While covered, plant growth is chlorotic and tender. Such growth is severely burned as it breaks through the soil surface under exposure to excessive radiation. The central stem of the plant is frequently lost and development is entirely from lateral branches at nodes beneath the soil. If infection develops at the point of injury, collar rot may progress down the stem to the crown and kill the plant.

A third type of specimen shows injury at a higher level on the stem than the others (Fig. 2). This may result if a large amount of soil is moved at one time onto a row of well branched plants of fair size. Such a method of cultivation tends to fold the plants in a manner that denies the stems the full protection of their leaves. On a severely bright day, even the older stems may be injured, if exposed at the soil level. Cankers on these older stems are generally much smaller than those on the young ones. As in other specimens, collar rot may follow injury. If observed at an early stage, the progression of the rot from the west to east exposure is obvious.

At the start of this study several points of similarity were noted between this disease and those such as "white spot" of coniferous seedlings (3), or "heat canker" of flax (5). These points of similarity were as follows: a) in respective fields, the location of the cankers on the stems was consistent among plants and was correlated with the soil level, either at the time of emergence or after cultivation; b) primary injury was consistently on the west to southwest exposure of the stem; c) a variable complex of saprophytic or weakly parasitic organisms was associated with the disease.

METHODS.—A series of exploratory observations was made with peanuts growing under a variety of circumstances in both the field and greenhouse. In these, special note was made of the temperatures of the air, in the soil about the plant stem, and on the plant stem. Under field conditions in Early, Baker, Dougherty, and Worth counties, Georgia, meteorological data were included.

All temperatures were determined by use of a 30 gauge copper-constantin thermocouple and potentiometer. This portable equipment was frequently checked against standard equipment at field stations of the U. S. Weather Bureau. Errors were not found to exceed $\pm 0.25^{\circ}$C. Air temperatures were taken in the field under the shade of the open cover of the potentiometer. Soil temperatures were measured by burying the thermocouple at the indicated depth in the soil. Reference to temperatures in the soil surface means the thermocouple was just covered in the surface soil rather than merely in contact with the surface. Estimates of temperatures in the plant stem were made by momentarily pressing the thermocouple against the plant stem.

An artificial source of radiant heat was used with peanuts growing in greenhouse culture. This was a 250 watt "heat lamp" with a clear glass bulb and silvered reflector. Exposure under such a lamp was assumed comparable to, and within the range of solar radiation if the following condition was fulfilled. The rate of change, or the degree of temperature must not exceed that observed in the field with the same soil in a comparable physical condition. In order to obtain the desired exposure with this equipment, the distance between the base of the plant and the lamp was seldom greater than 18 in. When the physical laws of radiation are operating within such a short distance, relatively small differences in proximity of the lamp to specimens or parts of specimens mean relatively large, perhaps critical differences in exposure. For this reason, it was found necessary to protect the upper parts of the plants with a cardboard baffle.

FIELD OBSERVATIONS.—One of the most outstanding phenomena observed in field studies and farm plantings was the correlation between the condition of the sky and variations of temperature in the surface soil.

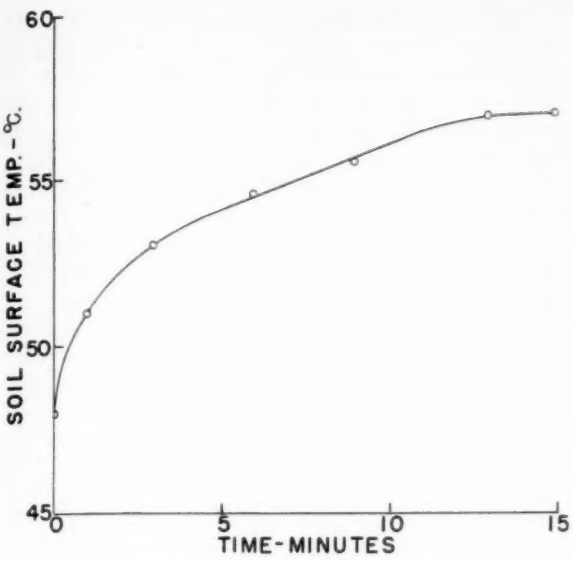

FIG. 3. Rapid rise in temperature of the surface soil during a period of 15 minutes following the passage of a small cloud in the sky. Air temperatures varied between 33°–34° C.

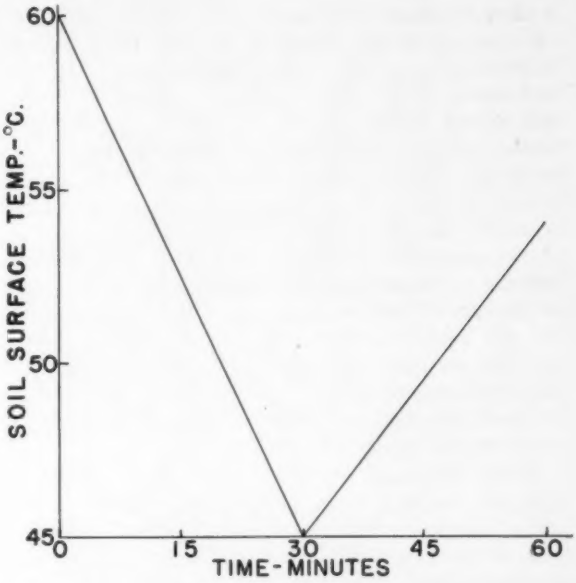

FIG. 4. An example of the range of temperatures found in the soil surface during a period of 1 hour while air temperatures remained relatively constant between 33° and 34° C.

Under certain conditions, the temperature in the soil surface changed rapidly and frequently over a relatively wide range. Temperatures were noted to increase from 48° to 57°, or 9°C., within 13 minutes, and decrease from 60° to 45°, or 15°C., within 30 minutes (Figs. 3, 4). Such changes are of greater magnitude than usual, but in no sense unique in the area studied. While such changes of temperature occurred in the soil surface, air temperature remained relatively constant, within a range of 1 degree.

These changes were correlated with the passage of a small cloud in the sky. The type of cloud most frequent during periods of fair weather in the area studied is called a "fair weather cumulus"[2]. A few small clouds of this type usually appear in the sky about 11 A.M., and increase in numbers until about 3 P.M. They are typical of fair weather during the spring and summer when radiation develops convection currents of sufficient magnitude in the lower atmosphere. They are considered cumuli without vertical development. After temperatures reach the top of the diurnal curve, such clouds slowly disappear. As exemplified by their effect on temperatures in the soil surface, these thin clouds act as very efficient filters of radiant heat without causing readily apparent shade or changes in air temperature. The protection they afford young tender plants against radiant burning heat is seldom appreciated. During intervals without the protection of a cloud, temperatures in the soil surface commonly reached between 50° and 60°C. After midday, such periods of full exposure were seldom longer than 10–15 minutes.

[2] Correspondence, Frank P. Lamb, Meteorologist, U. S. Weather Bureau, Albany, Ga.

Heat cankers may readily appear on peanuts after a clear day without clouds to protect intermittently the plants from solar radiation. This was observed in a field of peanuts growing in Worth County. Note was first made of this field as it was being cultivated. No evidence of injury was found on any plants. In cultivation, large amounts of dry, dusty, somewhat sandy soil were being moved onto the rows. Such dry soil appeared to flow from the implements and formed a smooth, dry surface as it settled about the plant stems. Larger plants were left more or less bundled up without full protection of their foliage. Smaller plants of late emergence were practically covered by the soil. The following day was clear and bright, without a cloud in the sky. By 1:10 P.M. the temperature in the surface soil of this field had reached 62°C. The temperature was 55°C. in the soil at a depth of ¼ in. These temperatures were maintained for 90 minutes. During this interval, air temperatures increased from 32.5° to 35°C. No injury became evident during the exposure but, within 48 hours, small cankers were evident at the soil level on many stems. Growing points and partly covered leaves of the smaller plants were badly burned.

The importance of a leaflet as shade to a stem was well illustrated in the above field. As compared to 62°C. in the exposed soil, a temperature of 52°C. was maintained in the small shadow of a leaflet. The relatively abrupt gradient between the shaded spot and the surrounding soil indicates that in such dusty soil, relatively little heat was exchanged between these areas by conduction.

Heat canker has been found on fewer plants and to be less severe in farm plantings of peanuts with rows

in an east-west as compared to a north-south direction. All cases of severe injury to the crop have been in fields or parts of fields with north-south rows. In east-west rows, plants afford a certain amount of protection to one another if not too widely spaced. In a north-south row, each plant is dependent on its own leaves for protection from the afternoon sun.

A specially designed field plot was prepared by hand in Baker County. This included different varieties of peanuts, different dates of seeding, and different schemes of seeding and cultivation that might tend to increase or reduce exposure of the plant stems at the soil level. No significant amount of heat canker or collar rot occurred in this plot. However, between the small and varied parts of the plot, there was opportunity for pertinent observations not possible between widely separated locations.

Little difference was found between the temperatures in a crusted, dusty, or finely clodded soil surface under cloudy conditions. However when these were fully exposed to a bright sun, the highest temperature was consistently found in a dusty surface of loose, fine soil particles. The difference between a dusty and a crusted surface ranged up to a maximum of 9°C. Temperatures in a crusted surface, formed after a heavy shower, were comparable to those found in the compacted surface of implement tracks. The cooling effects of moisture well beneath the surface is more apparent in crusted or compacted than in dusty surfaces. Under relatively dry conditions, the crusty surface was warmer than a finely clodded surface, where both aeration and the amount of exposed surface per unit area were greater.

Comparisons were made between temperatures in soil surfaces with different angles of exposure to the sun. Surfaces forming a 90° angle to the direction of the sun were warmer. Under ordinary conditions of field culture, differences in surface temperatures due to this factor were small and of very minor importance, if any, in the occurrence or absence of heat canker. However, the angle of exposure as it might effect the direction of reflected radiation to the plant stem, is perhaps more important than its effect on the soil surface itself.

Air movement had a very noticeable cooling effect in the soil surface. At times, in mid- or late afternoon under conditions of severe exposure, slight impulsive air currents developed near the soil surface. During calm periods temperatures rose as much as 7°C. in 5 minutes.

From limited observations, it appeared that heat canker and collar rot are more prevalent in Runner than in Spanish varieties. This is perhaps due to the manner in which the seedlings emerge. Leaves on Spanish seedlings unfold very readily and thus reduce the chance for severe exposure of the stem at this tender stage of development. Runner seedlings extend from the soil much further before their leaves spread.

No evidence was found to indicate a direct correlation between temperatures in the surface soil and the occurrence of heat canker. Temperatures obtained at a fixed location in the soil surface were a measure of variation in amounts of solar radiation reaching the ground level, and not a measure of how much heat was reaching or being absorbed by the plant stem. No singular factor in the environment was found to be a consistent basis for prediction of injury. A complex of several variables is apparently required to accomplish injury. A technique for continuous measurement and integration of all these factors is required to describe completely and accurately how and when injury will occur. These problems of technique were well beyond the scope of the present study.

GREENHOUSE TESTS WITH ARTIFICIAL RADIATION.—Field observations were confirmed in tests with artificial radiation under greenhouse culture. Under these conditions with better control of environmental factors, it was more evident that no measure of temperature in the environment of the plant was a satisfactory basis on which to predict the occurrence of injury. The best basis for prediction of injury was obtained by momentarily pressing the thermocouple against the lower part of the stem during periods of exposure to artificial radiation. If a reading of 45°C. was obtained, macroscopic injury was likely to be apparent within 24 hours. If a reading of 50°C. was obtained, injury was certain and likely to be lethal. It is noteworthy that this range of temperature, 45°–50°C., is the same as generally determined to be lethal for plant protoplasm (4). If exposure of the plants was continued until any macroscopic change became evident in the plant stem, the plants were dead within 24 hours.

Some characteristic, variable between plants, appears ultimately to determine the exposure required to cause respective degrees of injury to the plant. Variations in the occurrence or severity of injury were not correlated with factors such as slight differences in growth, or location of plants within the arrangement of equipment. Likewise, there was no correlation between these factors and the temperatures determined by momentarily pressing the thermocouple against the stem near the soil level. These factors, apparent from the environment of the plant, either exist only in theory, or are completely obscured by some greater factor within the plants themselves. The difference between the plants in a single pot was evidence that different amounts of heat were required to obtain comparable changes in the surface temperature of respective plant stems. This inherent character of the plant is comparable in effect to the physical property, "specific heat".

Tests were made in the greenhouse to confirm an observation that heat canker was more prevalent and severe in fields where the soil had been in a finely powdered dry condition. The surface of the soil was puddled and a dry crust was allowed to form about the bases of the stems. Later this crust was left un-

TABLE 1.—*Comparison of heat canker obtained by artificial radiation on plants surrounded by a dusty or crusty soil surface*

Soil surface	No injury	Severity of canker slight	severe	lethal
Crusty	27	3	2	2
Dusty	14	6	7	7

disturbed in ½ the area of the pot, while the other half was worked into a finely powdered smooth surface. This allowed identical exposure of the respective halves of a plant population, ½ with a crusted, and ½ with a dry powdery soil about the bases of their stems. After exposure under the "heat lamp", heat canker developed both on a greater number of plants and to a generally greater degree of severity where the soil surface was dry and powdery (Table 1). Here again, as in the field, moisture within 2 in. of the surface had a greater cooling effect on crusted as compared to the dusty surfaces.

Both the absorptive and reflective powers of the dusty soil surface may contribute to the development of heat canker. Neither of these factors could be well separated and defined in this study. The greater temperature in the dusty, as compared to the crusted, surface of the same soil indicates a greater absorption of solar radiation. However, this was no measure of how effectively this heat was transferred to the plant stem. The rather abrupt gradient of 10°C. between the fully exposed soil and the small shadow of a leaflet indicated a rather minor role for this factor. After cultivation, the dusty soil in some fields settled with a very smooth surface that appeared highly reflective. No facility was available to measure the transfer of heat by reflection. However, the potentiality of this factor was demonstrated by spreading aluminum foil on the soil about the base of the stem. Care was taken that the foil was close to but not touching the plant stem. If exposure under artificial radiation was severe enough to injure any of the plants without foil, those with foil were usually killed. Under less severe exposure, plants with foil showed some injury whereas no injury was evident on those without foil. In such tests, both the foil and soil remained well below lethal temperatures. Thus without contact with a hot surface, injury at the base of the plant was entirely the result of direct and reflected radiations.

In greenhouse tests, collar rot frequently followed heat canker. The infection occurred naturally without inoculation or incubation and was comparable in every respect to specimens collected from field plantings. Attempts to reproduce this disease have been generally unsuccessful (2). This is probably due to the fact that inoculation with a scalpel, needle, or abrasive, leaves very little morbid tissue intact at the wound. In contrast, a lesion of burned tissues, more or less intact, offers a much more favorable medium for entry and progressive development of weakly parasitic or saprophytic organisms.

DISCUSSION.—These studies with peanuts extend the concept of how heat canker is accomplished. The observations made here with peanuts agree with a previously reported observation that "canker occurred most abundantly where the bases of the young flax plants and the adjoining soil were exposed to intense sunlight" (5). With flax in this previous study, it was concluded, "Evidence indicates that heat canker of flax results from a combination of succulence in the young flax plants and high temperatures of the soil surface in immediate contact with the succulent stem tissues." This conclusion is too limited to be tenable in this study with peanuts.

In these studies with peanuts, susceptibility appears to be more than succulence. This was exemplified by the differences in injury between plants of the same age and appearance growing less than an inch apart under controlled culture.

High soil temperatures are more of a coincidence than a full explanation for development of heat canker. The greater incidence of canker with plants having a warmer dusty soil, instead of a cooler crusted surface about their stems, might lead one to correlate soil temperature with injury. However, as shown by the extreme tests with aluminum foil spread over the soil, both direct and reflected radiation must be considered as potentially injurious factors. The lack of accurate measure and sufficient control of this factor for reflection may in part explain the lack of correlation between soil temperatures and injury in replicated tests.

With respect to the environment in which heat canker occurs, the primary consideration must be the transmission of heat rather than degrees of heat. The result of this heat will depend upon the response in the respective plant stems to each unit of heat absorbed. Until facilities are developed to measure and control the various factors effecting heat transmission, the manner by which injury occurs must be deduced from qualitative responses in the plants and their environment.

By integrating the observations made at various times and places in this study, the following deductions are made. The surface soil may reach a temperature of 60°C. for 30 minutes at about midday. Because of the location of the sun in its orbit at this hour, an insignificant amount of radiation strikes the stem directly or is reflected to it. Under these conditions, the temperature of the stem is conditioned by the air and the soil and no injury results. Two or 3 hours later the same degree of temperature may be obtained in the soil for a comparable period of time. The base of the stem is not only in contact with the soil, as at midday, but, due to the angle of incidence to the sun, is exposed to both direct and reflected rays. The first stages of wilt are often apparent in the plant by this time of day. This indicates a reduced moisture content or transpiration stream that might reduce the speed of heat exchanges within the plant and favor

a localized accumulation of heat. Thus, the addition of direct and reflected radiation to the stem, and a change in the physiological status of the plant may result in injury.

No recommendations for control of these diseases can be offered with complete assurance. On the basis of data gained in this study, chances of injury should be reduced by arranging the rows so that young plants offer one another as much protection from the afternoon sun as possible. At the latitudes of North Dakota, north to south rows were recommended for flax (5). In the southern area of Georgia, severe exposure is on the west to southwest. Observations so far have been that canker is more severe on peanuts in north to south than in east to west rows. Cultivation should be timed to avoid in so far as possible dusty, highly reflective soil surfaces about the base of the plants. A finely clodded surface is most desirable.

SUMMARY

Heat canker and collar rot of peanuts are defined as 2 separate diseases. Heat canker is limited to injury of the plant stem by radiation at the soil surface. Collar rot is used as a somewhat generic term to include a primary injury of the stem by heat or other causes, and secondary infection by weak parasites.

In the southern area of Georgia, heat canker and collar rot have been erratic in occurrence and variable in severity on peanuts.

Lesions of heat canker are consistently at the soil level on the west to southwest exposure of the plant stem.

Typical specimens of heat canker and collar rot were obtained in greenhouse culture by exposure of peanut plants to the rays of a heat lamp. In such cases, all soil conditions and temperatures of the environment were within the range of those observed under natural field conditions.

Heat canker occurs after the estimated temperature reaches 45°–50°C. in the plant stem at the soil level.

No temperature found in the environment of the plant during exposure to radiant heat could be used as a basis for prediction of heat canker.

The cause of heat canker, a quantitative phenomenon, is only indicated instead of proven and properly described by qualitative measurements such as degrees of temperature in the environment.

Data indicated that the chances of injury may be reduced by cultural practices which maintain a finely clodded soil surface about the base of the plants, and by planting rows in a direction by which young plants offer one another the most protection from direct exposure to the afternoon sun.

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A DISEASE OF BIRDSFOOT TREFOIL CAUSED BY A NEW SPECIES OF STEMPHYLIUM [1]

J. H. Graham [2]

A previously unreported disease of birdsfoot trefoil (*Lotus corniculatus* L.) caused by an unknown species of Stemphylium was found in the Northeastern United States in 1951.[3] The fungus attacks the aerial parts of plants causing girdling of stems and premature defoliation. In a survey of forage crop diseases in New York in 1952, Roberts et al[4] found the disease in all fields examined and estimated 1 and 1.3 per cent losses in the first and second cuttings, respectively. Wider use of birdsfoot trefoil may increase prevalence and importance of the disease. Results of studies of the disease and its causal agent are reported in this paper.

SYMPTOMATOLOGY.—Leaves, stems, pods, and seed are attacked by the pathogen (Fig. 1). Young leaf lesions are reddish-brown, slightly sunken, and round or when at the leaf margin, semicircular. The spots increase in diameter up to 5 mm., become darker and may be concentrically zonate. Centers of mature lesions are lighter than the surrounding borders. Usually only 1 lesion causes a leaflet to drop prematurely. Under some conditions leaves and stems show tiny (approximately 1 mm. diameter), reddish-brown dots which never increase in size.

Copper-colored cankers outlined by a watery border form on the stems; they vary from dots to boat-shaped areas 25 mm. long. The centers of large lesions are ashy gray and under moist conditions become partially covered with black spore masses. Lesions often develop under diseased leaves which adhere to the stems. Young stems may be girdled and killed back to the crown.

On green and ripening pods the fungus causes irregular dark brown blotches and often shrivels and discolors the seed.

IDENTITY OF THE FUNGUS.—In nature the fungus sporulates readily on diseased tissue. Conidiophores arise singly or less often in fascicles of 2 or 3. On overwintered stems, clusters of conidiophores, rarely branched, arise from black stromatic bodies as well as directly from the epidermis. Conidiophores are golden to dark brown, somewhat darker at the bulbous tips, septate, 27–66 × 4–10 (average 49 × 9) μ with the tips and sometimes the basal cells approximately 2μ wider; in culture they may reach a length of 150μ. Buff to olive brown muriform conidia are borne singly, have rather smooth walls, are commonly spherical to rectangular with a prominent central construction (Fig. 2). Often one end of the spore is slightly larger than the other. The spores fall within the range of 21–37 × 14–33 (average 30 × 21)μ but average 2μ shorter in culture.

FIG. 1. Symptoms incited by *Stemphylium loti* on leaves and stems of birdsfoot trefoil.

[1] Accepted for publication July 2, 1953.

Contribution No. 124 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the twelve Northeastern States.

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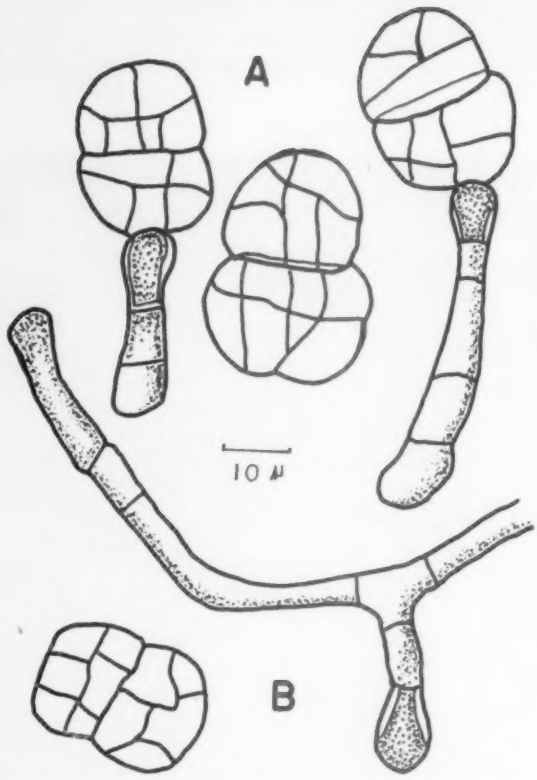

FIG. 2. Conidia and conidiophores of *Stemphylium loti*. A) from host tissue; B) on oatmeal agar.

In nature, numerous black sub-epidermal, erumpant stromatic bodies measuring 159–424 × 100–212μ are produced on dead stems of birdsfoot trefoil in late fall and winter. Under moist conditions in the spring a large number of conidiophores grow from the stroma (Fig. 3).

The fungus grows poorly on potato-dextrose agar but on oatmeal agar produces large cheatura, drab to dark grayish olive,[5] powdery, appressed colonies. Young colonies have wide white margins which later darken; after 8–10 days the centers develop zones of lighter gray mycelium. Abundant sporulation occurs on both media. On sterile alfalfa and birdsfoot trefoil stems the fungus produces little mycelium but an abundance of spores with many small stromatic bodies, 30–45μ in diameter, at the base of the stems in the water.

The sexual stage of the fungus has not been found.

The only previous account of Stemphylium on birdsfoot trefoil found in the literature according to Neergaard[6] was by Rostrup, who reported *Macrosporium globuliferum* Vestergr. on stems of *Lotus corniculatus*. Neergaard, however, examined the remaining herbarium material and identified it as *Pleospora herbarum* (Pers.) Rab. (*Stemphylium botryosum* Wallr.). The fungus described herein differs from the latter species primarily in possessing a smooth or slightly roughened conidial wall (not spiny), no evidence of a sexual stage, and it is restricted to species of Lotus.

The Stemphylium on Lotus is similar to *S. sarcinaeforme* Cav. which causes a leafspot of red clover. Conidia and conidiophore measurements differ slightly but fall within the same range and the conidial walls of *S. sarcinaeforme* are smooth while those of the Stemphylium on Lotus are smooth or roughened. Conidiophores and conidia of the fungus on Lotus are lighter colored than those of the clover fungus and never become fuliginous or black. Furthermore, isolates of Stemphylium from trefoil produce stromatic bodies whereas those of *S. sarcinaeforme* do not. In addition, each pathogen is limited to the host on which it was reported. On oatmeal agar colonies of *S. sarcinaeforme* are olivaceous black in contrast to those of the Lotus fungus which are lighter colored as described above.

Because of these morphological and physiological differences the fungus is described as a new species, and the following name is proposed: *Stemphylium* **loti** sp. nov.

Maculis foliorum rotundis, semicircularibus in margine, 1–5 mm. diam., subrubris vel fusco-brunneis, saepius pericentrice zonatis; lesionibus in caulis cupreis, centro cano, ad 25 mm. longis; areis irregularibus et brunneis in valvulis; conidiophoris singularibus, rare 2–3, non-furcatis, aureis vel fusco-brunneis, fuscioribus ad bulbosam apicem, 2–7 septatis, 27–66 × 4–10μ, 6–11μ ad apicem; conidiis luteis vel olivo-brunneis, muris glabris vel rugosis, muriformibus,

FIG. 3. Stromatic bodies bearing conidiophores and conidia on overwintered stem of birdsfoot trefoil.

[5] Ridgway, R. 1912. Color standards and color nomenclature. Washington, D. C.

[6] Neergaard, P. 1945. Danish species of *Alternaria* and *Stemphylium*, 560 pp. E. Munksgaard, Copenhagen.

quadratis, centro constricto interdum globoso, 21–37 × 14–33μ, plerumque 30 × 21μ. Corporibus stromaticis in siccis caulis repertis, nigris, subepidermalibus, erumpentibus, rotundis vel ovalibus, base plana, 159–424 × 100–212μ. Conidiophoris ex corporibus stromaticis verne erumpentibus. Hab. parasitice in foliis, caulis, valvulis *Loti corniculati* L.

Type locality: Three miles south of Chittenango, New York.

Effect of temperature on the fungus in culture.—The cardinal temperatures for mycelial growth were determined by measuring the diameter of colonies on potato-dextrose agar and oatmeal agar and by weighing mycelial mats in potato-dextrose-yeast extract liquid media. After 8 days maximum growth occurred at 24–28° C. The mycelium failed to grow at 2° and 37° C. Best sporulation occurred from 19–25° C. on potato-dextrose agar and near 22° C. on oatmeal agar. In contrast, the optimum temperature for mycelial growth of *S. sarcinaeforme* on potato-dextrose agar after 8 days was near 22° C. and some growth was observed at 2° and 37° C.

By suspending conidia of the fungus in drops of water on glass slides for 4 hours, the optimum temperature for germination was found to be 21–28° C., with insignificant differences within this range. At the optimum temperature more germ tubes of greater length were formed.

Pathogenicity.—The Stemphylium disease of birdsfoot trefoil occurs throughout the growing season in the northeastern states with most damage occurring during rainy periods in the summer. A large amount of leaf-spotting is seldom observed at any one time because of rapid defoliation. Several days of moist weather are necessary for infection and girdling of stems.

Artificial inoculations were conducted to determine the optimum temperature for infection and disease development. Inoculations were made by lightly spraying a spore and mycelial suspension on actively growing plants. The inoculation chambers were held at 18°, 22°, 26°, and 29° C., at 100 per cent relative humidity and under approximately 500 foot-candles for 16 hours each day. Most damage occurred at 26° C. while moderate infection was evident at the other temperatures.

Cross-inoculation and host range studies were conducted in conjunction with the temperature tests. Isolates of Stemphylium from birdsfoot trefoil collected in the northeast failed to infect alfalfa, alsike, Ladino, and red clovers. Virulent cultures of *S. sarcinaeforme* and *Pleospora herbarum* did not attack birdsfoot trefoil.

Sixteen strains or varieties of 4 species of Lotus were inoculated in the greenhouse with isolates from birdsfoot trefoil. *L. scoparius* (Nutt.) Ottley and *L. tenuis* Waldst. & Kit. ex Willd. were susceptible whereas 3 selections of *L. uliginosus* Schk. (New Zealand and 2 varieties, Beaver and Columbia) were slightly resistant. The following varieties and sources of *L. corniculatus* were inoculated: Granger, Empire, European, Viking, Leofoil, and Cascade varieties, 2 selections from Italy, and introductions PI 196539, PI 202383, and PI 202700. Only Granger showed a small amount of resistance whereas the other plants were moderately to severely attacked.

Overwintering.—The fungus survives from season to season on host tissue and in association with seed. In late fall and winter black stromatic bodies develop on dead stems and in the spring produce numerous conidiophores and conidia. In addition, clusters of conidiophores arise directly through the epidermis of the stems.

The fungus has been isolated from immature seed and from seed harvested from fields in which the disease was present. Although 4 per cent of the seed of 1 lot from New York were infected with Stemphylium, the percentage usually was less than 1.

Young seedlings may be killed by the fungus growing from the cotyledons into the stem. Abundant sporulation has been observed on diseased and dead seedlings in the field.

SUMMARY

A disease attacking the aerial parts of birdsfoot trefoil was studied and the causal agent identified as *Stemphylium loti* n. sp. Optimum temperature for growth and infection was 24–28° C. Isolates of Stemphylium from Lotus failed to infect alfalfa, alsike, Ladino, and red clovers and isolates from alfalfa and red clover did not attack *L. corniculatus*. The fungus overwinters in association with seed and as mycelium and stromatic bodies in plant tissue. No highly resistant plants were found.

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STUDIES ON THE INHERITANCE OF RESISTANCE TO MYCOSPHAERELLA BLIGHT IN GRAM [1]

Abdul Hafiz and Muhammad Ashraf

Cicer arietinum L. (gram or chick-pea) is often attacked by a blight disease caused by the fungus *Mycosphaerella rabiei* Kovacevski (*Ascochyta rabiei* (Pass.) Labrousse), with heavy losses. Previous work has shown that 2 exotic gram types of French origin obtained from the Bureau of Plant Industry, Washington, D. C., are highly resistant.

MATERIALS AND METHODS.—The 2 blight resistant French types, F8 and F10, and 2 susceptible Punjab types, P7 (one of the classified Punjab types isolated from local material and commonly grown by farmers) and C7 (from a cross between P7 and a Kabuli variety, *Cicer kabalicum*) were used. Large samples of each parental type were tested for reaction to blight under artificial conditions of inoculation, as well as F_1 progenies of F8 × F10, P7 × C7, F8 × P7, F10 × P7, their reciprocals, F_2 and F_3 progenies of F8 × P7, and its reciprocal.

Mycosphaerella rabiei, which according to Luthra et al (1939)[2] has only 1 physiologic form in the Punjab, was isolated from single spores on freshly blighted twigs and cultured on oat-meal agar. Cultures were grown in conical flasks for 20–25 days at 25° C. Then the fungal growth containing pycnospores was scraped, mixed in sterilized water and strained through muslin; the concentration was standardized at 20–30 spores per microscopic field under high power. Plants were sprayed with the spore suspension 3 times at 10-day intervals.

EXPERIMENTAL RESULTS.—*Behavior of parents.*—One hundred plants of each parental type were tested under artificial inoculation with the gram blight fungus. Type F8 and F10 proved highly resistant, with average disease indices of 4.32 and 4.97, respectively. The other types P7 and C7, proved highly susceptible, with average disease indices of 93.50 and 95.50, respectively (Table 1).

Behavior of F_1.—The maximum number of F_1 plants available in each cross varied from 3–17 and they were tested for reaction to blight under artificial inoculation. The results are in Table 2. From the data it can be seen that susceptible × susceptible crosses yielded susceptible progeny whereas resistant × resistant and resistant × susceptible crosses yielded resistant progeny. It is clear that resistance to blight is dominant. Furthermore, there is no difference in the behavior of direct and reciprocal crosses with respect to blight resistance.

Behavior of F_2.—F_2 progeny consisting of 810 plants from 8 F_1 plants derived from the cross F8 (resistant) × P7 (susceptible) and its reciprocal were tested for reaction to the blight fungus (Table 3). A total of 606 F_2 plants were resistant (0–50 per cent infection class) and 204 susceptible (51–100 per cent class). This gives a 3:1 ratio with a P value of 0.95–0.50, indicating that the resistance is controlled by a single

[1] Accepted for publication July 3, 1953.
The authors are indebted to Dr. A. Sattar, Plant Pathologist, for providing some of the material.

[2] Luthra, J. C., A. Sattar, and K. S. Bedi. 1939. Variation in Ascochyta rabiei (Pass.) Lab. the causal fungus of blight of gram, Cicer arietinum L. Ind. Jour. Agri. Sci. 9: 791-806.

TABLE 1.—*Disease indices representing reaction of parent types to blight*

		Number of plants observed in each class								
		A			B	C	D	Total		S.E.
Type	Year	0%	1-5%	6-15%	16-50%	51-90%	91-100%	population	Mean	(E)
F8	1949-50	10	69	21	....	....	....	100	4.32	0.33
F10	1949-50	8	63	29	....	....	....	100	4.97	0.36
C7	1949-50	....	....	....	....	....	100	100	95.50	0.00
P7	1949-50	....	....	....	....	8	92	100	93.50	0.67

TABLE 2.—*Disease indices representing reaction of F_1 progenies to the blight fungus*

		Number of plants observed in each class								
		A			B	C	D	Total		S.E.
Cross	Year	0%	1-5%	6-15%	16-50%	51-90%	91-100%	population	Mean	(M)
F8 × F10	1950-51	....	8	....	....	....	....	8	3.00	0.00
P7 × C7	1950-51	....	....	....	....	....	8	8	95.50	0.00
F8 × P7	1950-51	2	6	....	....	....	....	8	2.25	0.40
P7 × F8	1950-51	....	6	....	....	....	....	6	3.00	0.00
F10 × P7	1950-51	6	11	....	....	....	....	17	2.12	0.24
P7 × F10	1950-51	2	1	....	....	....	....	3	1.33	0.68

TABLE 3.—*Disease indices representing reaction of F_3 progenies to the blight fungus*

Cross	Number of plants observed in each class				Total population	Mean	S.E. (M)
	A	B	C	D			
	0-15%	16-50%	51-90%	91-100%			
P7 × F8	115	25	28	16	184	28.2	2.25
F8 × P7	382	84	100	60	626	29.4	1.26
Total	497	109	128	76	810		

and independent pair of factors.

Behavior of F_3.—Eleven families, comprising 214 plants derived by self fertilizing resistant F_2 plants, were subjected to artificial inoculation tests. Three families remained resistant and 8 segregated, giving a 3:8 ratio of homozygous to heterozygous lines whereas a 1:2 ratio is expected if the inheritance is monogenic.

SUMMARY

Altogether 34 F_1, 810 F_2 and 214 F_3 plants (constituting 11 families) were studied, derived from crosses between 2 blight resistant types, F8 and F10 and 2 susceptible types, P7 and C7. All F_1 plants of the direct and reciprocal crosses between the resistant and susceptible types were resistant to blight, like the parents, showing that resistance is dominant. The cross between 2 resistant types gave resistant hybrids and that between susceptible types gave susceptible plants in the F_1 generation.

Segregation in F_2 progeny took place in 3:1 ratio, indicating that resistance is controlled by a single and independent pair of factors. The reaction of F_3 progeny also substantiated the monogenic nature of inheritance of resistance.

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PRELIMINARY REPORT ON PRODUCTION OF HYDROGEN CYANIDE BY A SNOW-MOLD PATHOGEN [1]

J. B. Lebeau [2] and J. G. Dickson [3]

Winter crown rot or snow-mold of alfalfa and other forage plants in Western Canada is caused by an unidentified low-temperature basidiomycete. Damage is associated with snow cover and it is most severe when the snow melts slowly in the spring[4]. Likewise, severe disease development under artificial conditions occurs only when the plants are in a confined atmosphere at relatively low temperatures, 2–4° C.

Investigations on the physiology of the fungus over a range of temperatures indicated the production of hydrogen cyanide (HCN), first detected from a culture of the fungus on soybean meal—soil substrate grown at 4°. Subsequent experiments showed HCN production by this fungus on both natural and synthetic media.

The fungus was grown on synthetic and plant tissue media. HCN production was compared on 3 nitrogen sources with equivalent concentrations of nitrogen (4 gms./l.). Casein hydrolysate, asparagine, and potassium nitrate were added to Richard's solution containing 12.5 gm./l. pectin as a carbon source. Non-aerated cultures in 125 ml. flasks were used and HCN was expressed in p.p.m. on the basis of the volume of the culture solution. The natural media were ground finely and 2 gms. dry material was mixed with 15 gms. of washed sand and 5 ml. of distilled water for each flask. The HCN was expressed as p.p.m. of the 2 gm. oven-dried sample. The fungus growing on soybean meal and alfalfa crown-tissue meal gave the highest concentration of HCN of any of the substrates tested (Table 1). The several media used indicated HCN production at different rates and quantities.

Relative rates of HCN production of 4 isolates of the fungus were determined at 8° C. on the above mentioned liquid culture using asparagine as the nitrogen source. These rates, determined as p.p.m. HCN on the basis of the volume of culture solution, are expressed on a comparative basis using an index value of 100 for the highest. The average comparative HCN synthesis of the 4 isolates of the fungus B50A, B24, B49, and B2 at 8° was 100, 50, 43, and 28, respectively.

With both synthetic and plant media a good correlation between mycelial growth and quantity of HCN was indicated. The mycelial growth of isolate B24 and HCN production from 2–20° indicated synthesis during the active growth of the mycelium rather than associated with autolysis of fungus cells as suggested

[1] Accepted for publication July 3, 1953.
Joint contribution from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada—Contribution No. 1280—and the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

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[3] Professor of Plant Pathology, University of Wisconsin.

[4] Cormack, M. W. 1948. Winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta. I. Occurrence, parasitism, and spread of the pathogen. Can. Jour. Res. C. 26: 71-85.

TABLE 1. *Production of hydrogen cyanide by the crown rot pathogen on several media at 4° C.*

Substrate	Age of Culture	Ave. Conc. HCN[a]
	Days	p.p.m.
Casein hydrolysate	20	20
Do.	30	98
Asparagine	20	72
Do.	30	58
Potassium nitrate	20	15
Do.	30	15
Wheat straw	20	34
Do.	30	36
Alfalfa crown tissue	20	700
Do.	30	700
Soybean meal	20	2500
Do.	30	2500
Silt soil	60	22

[a] HCN was determined by steam distillation into alkaline solution and determination of HCN concentration colorimetrically.[5]

by other investigators for several basidiomycetes.[6] Growth of the fungus and HCN synthesis occurred at 2–20° with no growth nor HCN synthesis at 24°. In these experiments the maximum growth and an average of 1250 p.p.m. HCN were obtained at 12° on the alfalfa-crown meal in 30-day old cultures.

The quantity of HCN produced in closed cultures of the fungus was above the lethal dosage for seedling and year-old alfalfa plants. Alfalfa seedlings 6 weeks old were killed in less than 6 hours when the rootlets were placed in water and the plants suspended in a closed container over an actively growing culture of the fungus on soil, alfalfa-crown meal medium at 4°. Using year-old, dormant alfalfa plants, 60 per cent were killed in 10 days under the same conditions. The control plants suspended in a similar manner over a culture of *Fusarium oxysporum* (Schl.) Snyder and Hansen var. *redolens* (Wr.) Gordon showed no damage.

Both dormant and vegetatively-active alfalfa plants were killed in soil artificially infested with the fungus when the surface of the soil was covered with a partial seal of nonabsorbent cotton and wax or cellophane. Soil temperature below the sealed surface was held at 4° and the air temperature in which the tops developed was 16°. When alfalfa-crown tissue was incorporated in the surface layer of soil as a medium for fungus development, 90 per cent of the plants were killed in 14 days. The diseased plant crowns averaged 1400 p.p.m. HCN, dry weight basis, and the surrounding soil-fungus mat 56 p.p.m. When a silt soil with relatively low organic matter was used as a medium, only 55 per cent of the plants were killed in 60 days. The diseased crowns averaged 16 p.p.m. HCN and the surrounding soil-fungus mat contained none.

Preliminary experiments to determine the lethal concentration of HCN in water were run at 4° with 2 species of *Medicago*. Dormant plants were suspended approximately 2 in. above the HCN solutions in closed vessels and strips of filter paper placed over the plant crowns with the ends of the strips hanging down into the solution. HCN concentration of $.99 \times 10^{-2}$ M killed 80 per cent of the crown buds of year-old Grimm alfalfa (*M. sativa* L.) plants in 10 days. A concentration of $.99 \times 10^{-3}$ M killed 40 per cent of the buds in the same exposure time. At the latter concentration, however, some buds, apparently less advanced at time of exposure, developed when the plants were placed in soil in a cool greenhouse. In year-old plants of *M. falcata* L. under similar conditions, the upper, fully-developed crown buds were killed, but the numerous bud primordia situated lower on the crown developed into shoots when the plants were transplanted into soil in a cool greenhouse.

SUMMARY AND CONCLUSIONS

The low-temperature basidiomycete responsible for extensive winter crown rot damage in parts of Western Canada produced HCN in concentrations sufficient to kill buds and crown tissue of alfalfa plants. The synthesis of HCN is associated with the isolate of the fungus and with the medium upon which the fungus is grown and apparently is correlated with the mycelial development of the pathogen. Maximum concentration of HCN was produced on media composed of soil and soybean meal and soil and alfalfa crown-tissues. The high tolerance of the fungus to cyanide suggests an unusual metabolic process in this microorganism. HCN production was high at low temperatures and dropped off sharply at 16–20°, which was near the maximum temperature for the growth of the fungus on the media tested. This is in marked contrast to the 25° reported by Robbins et al.[6] for another basidiomycete.

At concentrations of HCN near the lethal threshold for alfalfa plants, fully-developed crown buds are more susceptible to injury than bud primordia. Higher concentrations of HCN did not produce any differential response of tissues.

Similar investigations have not been conducted under field conditions. The greenhouse and laboratory experiments strongly suggest that disease development is directly dependent upon the production of HCN by the fungus and the confinement of the lethal agent in the close vicinity of the plant parts until specific tissues or the entire crown is killed.

LABORATORY OF PLANT PATHOLOGY, EDMONTON, ALBERTA
AND
DEPARTMENT OF PLANT PATHOLOGY, MADISON, WISCONSIN

[5] Boyd, F. T. 1938. The determination of factors influencing the amount of cyanide in Sudan grass. University of Wisconsin Ph.D. Thesis.

[6] Robbins, W. J., Anita Rolnick, and F. Kavanagh. 1950. Production of hydrocyanic acid by cultures of a Basidiomycete. Mycologia 42: 161-166.

Bacterial Spot of Broccoli.[1] M. A. SMITH AND G. B. RAMSEY.[2] In February, 1952, specimens of diseased broccoli from a carlot grown in the vicinity of Oceano, California, were received from the Fresh Food Products Inspection Service of the Production and Marketing Administration at Chicago, Illinois. The inspection showed that 25 per cent of the bunches in some crates were diseased.

Previously, several shipments of broccoli to Chicago had shown a similar condition considered to be due to infections by *Alternaria*. While numerous isolations from the broccoli in 1952 failed to reveal the presence of *Alternaria* sp., most of them yielded bacteria in abundance.

The disease is characterized by purplish-gray to black lesions, varying in size from mere pin points to circular and oblong spots .5 to 1 mm. in diameter, on stems, petioles, florets and leaves. (Fig. 1, A, B.) Infections on stems sometimes coalesce and form lesions 3–5 mm. in length. Lesions on leaves when viewed by transmitted light show a dark center surrounded by a halo (Fig. 1, C), resembling closely the type of leaf infection of cauliflower caused by *Pseudomonas maculicola* (McCulloch) Stevens.[3]

A bacterial spot of broccoli and rutabaga was reported from New Jersey in 1940, but the disease was not ascribed to *Ps. maculicola*.[4] Records of the Plant Disease Survey indicate that *Ps. maculicola* occurred

[1] Investigations conducted by the Division of Handling, Transportation, and Storage of Horticultural Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the Department of Botany, University of Chicago.

[2] Pathologist and Principal Pathologist, respectively, Bureau of Plant Industry, Soils, and Agricultural Engineering.

[3] McCulloch, Lucia. A spot disease of cauliflower. U. S. Department Agr. B.P.I. Bul. 225, 1911.

[4] Clark, Elizabeth. 1940. Outstanding diseases of economic crops in New Jersey in 1940. U. S. Dept. Agr. Pl. Dis. Reptr. 25: 291.

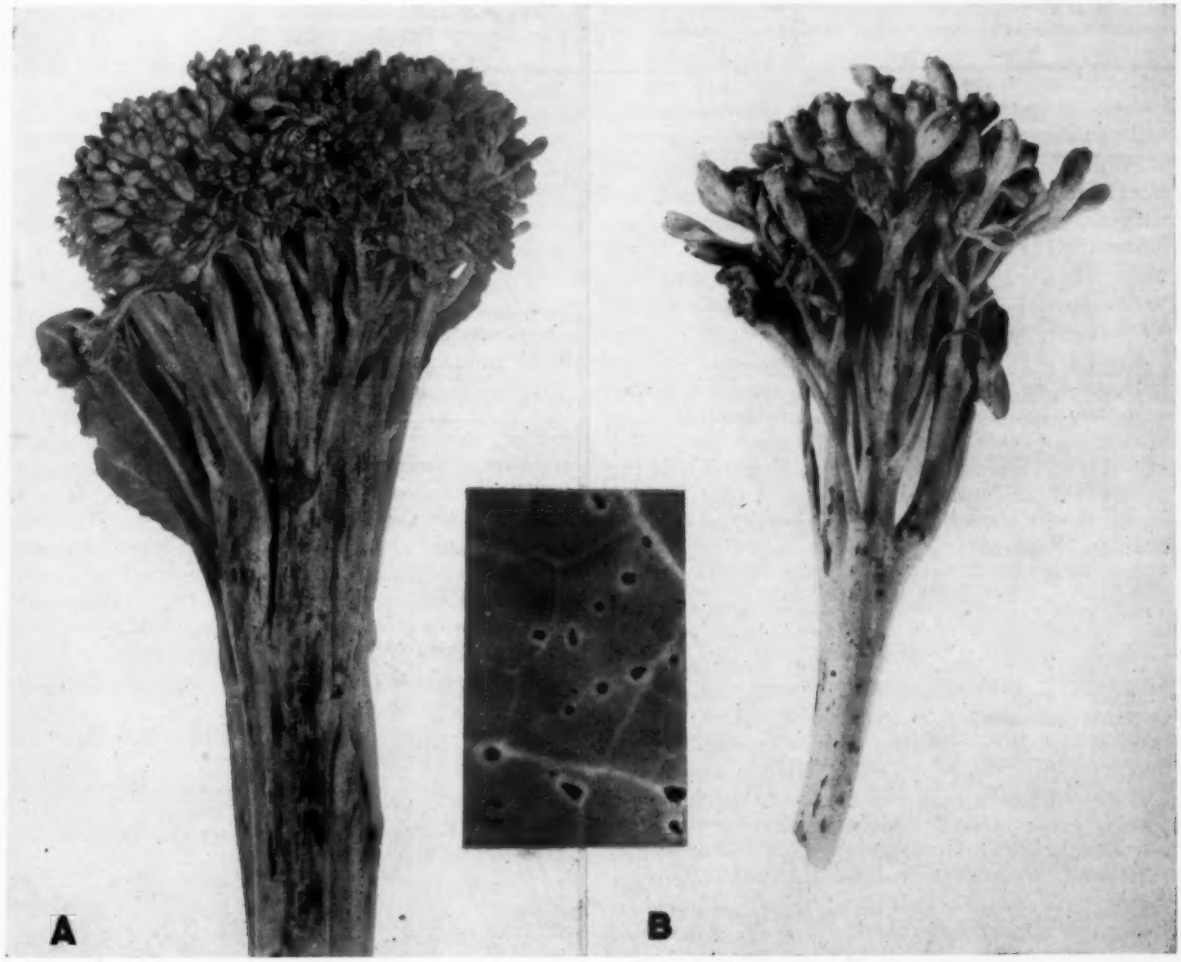

FIG. 1. Bacterial spot of broccoli. A and B) Infections on stems and florets. C) Water-soaked lesions on broccoli leaf.

on broccoli in San Mateo county, California, in 1943. There appear to be no other reports of the occurrence of a similar disease on broccoli.

Numerous cultures were made from affected stems, petioles, florets and leaves of broccoli from the above-mentioned shipment, by tissue plantings or dilution-plate methods. A large percentage of these yielded bacteria. The organisms were maintained on beef-extract-peptone-dextrose agar (pH 6.8). Single-colony cultures were purified by the dilution-plate method. In the studies herein described pathogenicity, morphological, biochemical and physiological tests were made on 6 of the broccoli cultures and on 2 pathogenic cultures of *Ps. maculicola* from diseased cauliflower obtained on the local market.

PATHOGENICITY.—Five broccoli isolates were used in the first series of inoculations. Bacteria from 24-hour-old cultures were suspended in sterile distilled water and atomized on uninjured surfaces of broccoli stems, petioles, florets, and leaves and on heads of cauliflower. Numerous infections characterized by minute, slightly water-soaked purplish-gray lesions were first observed on stems and petioles of broccoli after 7 days. Similar lesions were seen on florets of broccoli and cauliflower after 9 days. Broccoli and cauliflower heads sprayed with distilled water remained free of the disease. The organism was re-isolated in pure culture from the infected broccoli and cauliflower.

Further inoculations of broccoli and cauliflower were made with isolates from infected broccoli and with the cultures of *Ps. maculicola*. Most of the inoculations were successful, and the organism was reisolated in pure culture. The lesions produced were similar and of the type occurring naturally on broccoli and cauliflower on the market.

Results of studies of morphological and cultural characteristics of the broccoli isolates and *Ps. maculicola* show that they are similar if not identical. There are no significant differences in carbon metabolism of the broccoli isolates and *Ps. maculicola*. It is suggested that the broccoli isolates should be considered identical with *Ps. maculicola*.—University of Chicago, Chicago, Illinois.

The Conidial Stage of Pseudoplea briosiana on Alfalfa.[1] R. R. NELSON and M. F. KERNKAMP. Several monoconidial isolates of Stemphylium which conform completely to the description of *S. botryosum* Wallroth produced *Pseudoplea briosiana* (Poll.) von Höhnel both on artificial media and on the leaves of alfalfa plants. Hitherto, no conidial stage has been reported for the fungus causing Pseudoplea leafspot of alfalfa.

Rostrup[5] in 1898 described a leafspot of white clover caused by a fungus which he named *Sphaerulina trifolii*. The same fungus or similar fungi were subsequently reported on both alfalfa and the clovers, and these were given several different generic and specific names. Pollacci[4] in 1901 described a fungus on alfalfa and burr clover which he called *Pleosphaerulina briosiana*. In 1918 von Höhnel[2] transferred this fungus to the Pseudosphaeriaceae and named it *Pseudoplea briosiana* (Poll.) von Höhnel. Petrak[3] then changed the name to *Pseudoplea trifolii* (Rost.) Petrak, which is now the generally accepted name. *Sphaerulina trifolii*, *Pleosphaerulina briosiana* and *Pseudoplea briosiana* (Poll.) von Höhnel are listed as synonyms of *Pseudoplea trifolii* (Rost.) Petrak. The present writers, however, believe that *Pseudoplea brosiana* and *P. trifolii* should be considered as separate species. The 2 important characters that differentiate these species of Pseudoplea are: 1) *P. briosiana* (Poll.) von Höhnel has muriform septate ascospores and was reported in nature only on alfalfa and burr clover; 2) *P. trifolii* (Rost.) Petrak has nonmuriform septate ascospores and was reported in nature only on red clover and white clover. The name *P. briosiana* has been retained for the fungus used in these studies on alfalfa because Pollacci[4] was the first to report and describe it on this host, and because the fungi on red clover and alfalfa are henceforth considered as separate species.

In early July, 1952, the leafspot disease caused by *Pseudoplea briosiana* was epidemic on the second growth of alfalfa in several breeding nurseries at the Agricultural Experiment Station, Rosemount, Minnesota. At that time, only *P. briosiana* was isolated from the large lesions on the leaflets. Several days later, both *P. briosiana* and *S. botryosum* were isolated from the same lesions and from other similar ones. By late July *Pseudoplea* had disappeared completely and subsequently only the Stemphylium stage developed.

In the laboratory, perithecia and ascospores of *P. briosiana* developed in single-spore cultures of *S. botryosum* and, conversely, the conidial stage developed from single-ascospore cultures of Pseudoplea. When alfalfa seedlings were inoculated with ascospores in the greenhouse, punctate lesions appeared on the leaflets, similar to those described for Pseudoplea in the earlier literature. *S. botryosum* was never found in these lesions, but perithecia with mature ascospores developed on the leaves within 14 days. When the seedlings were similarly inoculated a few weeks later, the resulting lesions were at first punctate but enlarged to form lesions several mm. in diameter and similar in appearance to those observed in the field in mid July. Both the Stemphylium and Pseudoplea stages of the fungus were isolated from these lesions. As the plants

[1] Paper No. 2995, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Höhnel, Franz von. 1918. Über Pleosphaerulina briosiana Pollacci. Ann. Mycol. 16: 162-163.

[3] Petrak, F. 1921. Über Pleosphaerulina briosiana Poll. Ann. Mycol. 19: 28-29.

[4] Pollacci, G. 1902. Sopra una nuova malattia dell'erba medica. (Pleosphaerulina briosiana Poll.) Atti. R. Ist. Bot. dell Univ. Pavia 2: 7: 49-54.

[5] Rostrup, E. 1899. Mykologiske Meddekeser (VIII). Spredte Iagttagelser for 1897-1898. Bot. Tiddskr. 22: 254-276.

became still older and approached senility, ascospores inoculated onto the leaves did not cause infection.

In all of the writers' studies *S. botryosum* attacked alfalfa in all stages of development but it produced its perfect stage, Pseudoplea, only on plants in the flowering stage or later.

Following is a brief description on the Pseudoplea stage: Perithecia are 110-150μ in diameter, brown when immature and black when mature. The perithecium has a long neck with an ostiole 40-50μ in diameter. Asci are hyaline, ovate to elongate, and 85-125μ long; they are sessile and never more than 4 in a perithecium. The ascospores are hyaline and muriform with 3 to 4 transverse septa and 1 to 3 longitudinal ones. Segments with longitudinal divisions are bulged, thus giving a constricted appearance to the spore. Mature spores are from 13 $\times$ 31μ to 17 $\times$ 40μ.

The taxonomic position of *Pleospora herbarum*, given in the literature as the ascigerous stage of *S. botryosum*, is uncertain. In the present studies Pleospora never was obtained from Stemphylium. It is possible that what has been called *S. botryosum* actually comprises 2 species that are morphologically similar in the conidial stage but different in the ascigerous stage.—Department of Plant Pathology and Botany, Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minnesota.

REPORT AND ABSTRACTS OF THE SIXTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 35th annual meeting of the Pacific Division of the American Phytopathological Society was held in the Hotel Carrillo, Santa Barbara, California, June 17-19, 1953, in conjunction with the annual meeting of the Pacific Division of the A.A.A.S. Included on the program were 38 papers and a field trip to points of interest in the Santa Barbara area. The Phytopathologists' dinner on Wednesday evening, June 17, was attended by 96 members and guests.

The membership voted unanimously to combine the 1954 Pacific Division summer meetings with the A.P.S. meeting to be held in Colorado in August or September 1954 and as a group unanimously favored meeting at Estes Park. The division also plans to meet with the A.A.A.S. in Berkeley in December 1954 and wishes to extend an invitation to all members of the A.P.S. to meet with the Pacific Division at that time. The type of meeting to be held in Berkeley has not been agreed upon.

Officers elected for the year 1953-54 were: *President*, GILBERT L. STOUT; *Vice-President*, GEORGE A. ZENTMYER; *Councilor*, WILLIAM B. HEWITT and *Secretary-Treasurer*, ROY A. YOUNG have one more year of a two-year term.

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Verticillium wilt of cotton: Studies of possible seed-transmission. ALLEN, ROSS M. *Verticillium*, inoculated into cotton seeds may remain alive there for 17 months; such seeds germinate. A large number of inoculated seeds planted in soil produced no plants diseased at maturity. Injections of *Verticillium* into various plant parts were made. Since all tissues of inoculated bolls, when moist, supported growth of *Verticillium*, it is likely that a very small percentage of seeds may act in disseminating the disease. Acid-delinted, surface-sterilized seeds from Verticillium-infected locks were cultured and the pathogen recovered 6 months after harvest. *Verticillium*, producing conidia, was observed in the pith cavity of dead, mature stalks in the field and in stalks standing 28 days in water in the laboratory. Pure cultures of the organism may be obtained from the pith region by touching the agar covered tip of a sterile transfer needle to the infected pith and then streaking an agar slant.

Greasy blotch of carnation. BAKER, KENNETH F. AND LILY H. DAVIS. In glasshouses of the San Francisco Bay area, California, during 1952-53 an unusual disease severely injured several carnation varieties. The basal leaves and stems lost their glaucous waxy bloom and appeared greasy or shiny green; the middle leaves had greasy blotches with radiately fibrillose margins. Fibrillate wax-cleared patterns from germinating spores were visible with a hand lens on upper leaves, in contrast to smooth margined spray residue spots. Yellowish pimples developed at stomata in affected areas of stems and leaves. These symptoms were reproduced by culture inoculations. Under 72$\times$ magnification and critical lighting, sparse, black, tortuous conidiophores of *Zygophiala jamaicensis* Mason (Commonwealth Mycol. Inst. Mycol. Paper 13, 1945) were visible on all affected areas. They bore 2 two-celled conidia which adhered together during dispersal. The slender mycelium is superficial except for limited penetration at stomata. Under moist conditions tiny, thin, black, superficial stromatic masses are formed. Though present in numerous glasshouses, the disease caused economic damage only in those maintaining high humidity, or during wet winter months. Control should be achieved by eliminating these conditions. The fungus, originally described on banana leaves, occurs on commercial banana fruit in California. It has a wide host range, producing variable symptoms.

Life history of Pleospora sp. found on gladioli. BALD, J. G. Pathogenic and non-pathogenic strains of *Pleospora* may be isolated from lesions and healthy tissue of gladioli infected with Stemphylium leaf spot. In the field the conidial stage may develop both on lesions and on tissues that have senesced and died or been killed by other causes. No carryover on corms has been observed. The perfect stage can be found developing during winter on dead flower stalks and sometimes on dead leaves. The life history may include a stage in which the fungus germinates on the surface of gladiolus leaves, and forms thick-walled resistant stomatal nodules. This is accompanied by death of guard cells and possibly also adjacent epidermal cells. The fungus may remain dormant as nodules on the green leaf until it dies, then under favorable conditions produce a mycelium in the dead tissue and a crop of conidia. Pathogenic types of *Pleospora* on susceptible varieties, e.g., Chamouny, are able to penetrate through stomata and immediately form a mycelium in living leaf tissue. In this variety a pathogenic strain can produce extensive light brown to tan lesions on leaves of all ages, in contrast to the red spots with halos usually described as characterizing Stemphylium disease of gladiolus.

Correlation between natural spread of peach mosaic and age of infection in the donor tree. COCHRAN, L. C. AND L. S. JONES. Repeated occurrence of sudden outbreaks of rapid natural spread of peach mosaic in experimental plots has been associated with retention of diseased trees for more than 1 year. Where 272 peach nursery trees were planted in March 1950 adjacent to older trees diseased

for 2 years or longer, some of the nursery trees showed mosaic symptoms by June 1950, and 157 of them were diseased in the spring of 1951. Mosaic was nearly 100 per cent in the sections of the rows nearest the older trees. The diseased trees were removed in February 1951 and replaced by healthy nursery trees. These were inoculated in September 1951 and were diseased in 1952. Not a single case of mosaic was present in April 1953 in a row of 132 nursery trees planted in March 1952 immediately adjacent to the inoculated trees. Spread to nursery trees took place in another part of the plot planted next to trees which had been diseased for 2 years. Absence of spread near trees in the first year of disease and rapid spread near trees disease 2 years or longer indicate factors associated with age of infection which limit acquisition of the peach mosaic virus by the vector.

Black cane rot of Syngonium auritum. DAVIS, LILY H. A form of *Ceratocystis (Endoconidiophora) fimbriata* caused economic loss in propagation beds of *Syngonium auritum* (*Philodendron trifoliatum*) in a southern California nursery. The disease caused brown to black, water-soaked, girdling cane cankers which occurred anywhere on the plant, but most frequently on parts in contact with the soil, or through aerial roots at the petiole bases. Characteristic yellowing of the foliage was followed by subsequent death of affected leaves. Overhead watering and mechanical injury, such as cutting wounds, contributed to the spread of the disease, although wounding is unnecessary for infection. The fungus penetrated deeply into the cortical tissues and into the vascular system as reported for susceptible sweet potato varieties. Under moist conditions, perithecia form commonly on old cut stubs and on cankers. The causal organism is morphologically identical to *C. fimbriata*, the cause of black rot of sweet potato, but cross-inoculation studies showed that the 2 forms are physiologically distinct. Since sporulation occurs internally, hot-water treatment of the canes was necessary for eradication of the fungus. A temperature of 120°F. for 30 minutes eliminated the pathogen with minimum injury to plants.

Presence of an unidentified ninhydrin-reacting substance in peach leaves affected with Western X-disease. DIENER, T. O. Qualitative paper chromatographic determination of free amino acids in extracts prepared from peach leaves with symptoms of Western X-disease revealed beside aspartic acid, glutamic acid, asparagine, arginine, serine, glutamine, threonine, alanine, γ-amino butyric acid, valine, (iso-) leucine, and proline an unidentified substance, which, after ninhydrin treatment, gave a blue, rapidly fading spot with an R_F value of 0.90 in a phenol-water system and an R_F value of 0.32 in a collidine-lutidine-water system. Samples from 25 Western X-diseased peach trees (J. H. Hale, Rochester, Elberta varieties and Lovell seedlings) were collected from widely separated locations in the State of Washington, using leaves from unaffected portions of the diseased trees and leaves from apparently healthy neighboring trees as control samples. The unidentified spot was consistently found with extracts from leaves with symptoms of the disease; it was not found with extracts from 32 control samples. Neither could a spot in this position be observed when extracts from leaves of trees showing symptoms of girdling or nitrogen deficiency were tested.

Ecology and hosts of flyspeck of apple in California. DURBIN, RICHARD D. AND WILLIAM C. SNYDER. Flyspeck of apple, *Microthyriella rubi* Petrak, occurs in restricted sites in Santa Cruz County and apparently is endemic. Maturing ascocarps have been collected in April during and following apple blossom season, on stems and sometimes on leaves of the following incomplete list of wild hosts: *Acer macrophyllum, Arbutus menziesii, Lonicera hispidula, Rhamnus californica, Rubus parviflorus, R. vitifolius, Sambucus glauca;* and on discarded cull apple fruits in an orchard. Inoculations of young apple fruits with a single ascospore culture resulted in each case in a cluster of specks, or ascocarps. Thus, a single infection is not limited to the formation of a single speck as reported by Tehon. High humidity evidently is required for infection and maturation of ascocarps since flyspeck is most evident on hosts in the fog belt near moist pockets of coastal redwoods. Because of the broad host range of the fungus, the presence of wild hosts or discarded apple fruits adjacent to orchards provides important inoculum reservoirs in those local situations where environmental conditions favorable to disease development exist.

Surface mold and decay of Kadota figs. ENGLISH, HARLEY. One of the important factors limiting the production of Kadota figs in California is the development of surface mold and decay of the fruit during the latter part of the harvest season. The surface mold, which appears as smudgy areas, is due largely to 1 or more species of *Cladosporium* and *Alternaria*, whereas the shallow surface rot is caused almost entirely by *Alternaria*. Surface mold usually develops only on the upper and outer surfaces of the most exposed fruit, where the greatest condensation of moisture occurs. Alternaria rot also is generally more prevalent on the most exposed surface of the fruit. In an attempt to control these disorders, fungicidal sprays were applied early in September, when a trace of surface mold was present, and 2 weeks later when the mold was more pronounced. Two applications were better than 1 and the late spray was more effective than the early one. Where 2 applications of Manzate, ziram, zineb, Zinc Coposil, and Vancide 51 plus zinc sulfate were applied the disorders were reduced by approximately 50 per cent. Captan and Phygon were somewhat less effective, and Crag 341 was little better than the check. Manzate, Phygon XL, and Crag 341 were somewhat phytotoxic.

Phytophthora root rot of alfalfa. ERWIN, DONALD C. In 1952 an undescribed root rot of alfalfa was observed in Yolo, Solano, San Joaquin, and Fresno counties of California. The upper leaves of severely affected plants were usually yellow. The affected area on the tap root was water-soaked and reddish-brown. Later the lesion extended through the bark, into the wood, and eventually throughout the entire root. Above and below the brown lesion the woody tissue became yellow. A *Phytophthora* sp. was isolated from diseased plants. Healthy alfalfa plants, 18 days after being inoculated with the fungus, showed a root rot identical to that seen in the field. The fungus was reisolated from the inoculated plants. Globose oogonia, averaging 31μ in diameter, amphigynous antheridia, and non-papillate, ovoid to obpyriform sporangia were produced on sterile hemp seed in distilled water. Hyphal swellings were also present in abundance. Greatest radial growth on potato-dextrose and Difco corn meal agars occurred at 25–27°C. Occasionally slight growth occurred at 33° but none at 36°. The fungus is tentatively identified as *Phytophthora cryptogea.*

Controlled reinfestation of treated soil. FERGUSON, JOHN. A major problem following soil disinfestation is recontamination by disease organisms. Since the first organisms returning after treatment dominate the soil flora, an attempt was made to control reinfestation by introducing nonpathogenic organisms. Untreated soil which decreased the spread of *Rhizoctonia solani* yielded organisms which were tested as retardants. Species of *Chaetomium, Pestalozzia, Myrothecium, Penicillium, Trichoderma,* and an unidentified Botrytidae when added to flats of steamed, high organic, sandy loam mixture in the glasshouse retarded the spread of Rhizoctonia damping-off of peppers. The last 4 named prevented the development of damping-off when retardant and *Rhizoctonia* were added at time of seeding. The inhibitory action of *Myrothecium* sp. diminished after a month in flats of growing plants. Growth of pepper seedlings in flats inoculated with the above fungi was slightly to moderately retarded, the severity depending on the fungus used and the type and amounts of soil amendments added. Data of this type suggest the possibility of adding a group of organisms to soil, in order to prolong

and buffer their joint inhibitory action on pathogens under a wide range of conditions.

Curly-top infection in southern California potato seed stock fields. GIDDINGS, N. J. In August, 1952 tests on sugar beets proved the presence of curly-top virus in potatoes being grown for certified seed stock in the Rosamond, California, area. These tests, from plants and tubers, indicated that there was 8–10 per cent infection in this field. A month later tests from plants and tubers obtained from a potato field near Tehachapi also had curly-top infected plants. All tests were made from plants showing symptoms believed to be due to curly-top infection. Curly-top virus was obtained from less than 50 per cent of these plants. This suggests that the symptoms induced by leafhopper infection of the growing plant are not readily recognized under southern California field conditions. The virus obtained was similar to curly-top strain #12 that was first described from Prosser, Washington.

Sugar beet virus yellows infection influences subsequent curly-top infection. GIDDINGS, N. J. There are many areas in the western United States where both virus yellows and curly top occur. Several tests have indicated that a sugar beet plant that has been infected by virus yellows, has recovered from some of the symptoms, and has then been inoculated with curly top is more likely to become infected with curly top than a comparable healthy plant. The curly-top symptoms induced in such plants also appear to be more severe than in plants not diseased with virus yellows.

Physical-chemical properties of five strains of TMV. GINOZA, W., D. ATKINSON, AND S. G. WILDMAN. Five strains of TMV, which have been classified into 2 groups on the basis of biological, chemical, and serological properties, were analyzed for the following physical-chemical properties: 1) isoelectric point, 2) electrophoretic mobility, 3) ultra-violet absorption spectra. Strains in Group I consisting of U_1, U_2 and U_4 have identical isoelectric points sharply distinguished from strains in Group II consisting of U_3 and U_5. Strains of Group I have identical electrophoretic mobilities over a pH range of 4–8. The mobilities of strains of Group II are identical over the same range of pH but are lower than Group I. Ultraviolet absorption spectra of the 5 strains are closely similar except that strains of Group I have a clearly recognizable difference in absorption at 290 mμ. Classification of strains into Groups I and II by these additional physical-chemical criteria thus agrees in all respects with the classification proposed on the basis of other biological, chemical, and serological data.

Propagation of leafy cuttings of Prunus mahaleb with adenine and indolebutyric acid. GRAHAM, S. O. In the course of establishing apparently virus free clones for stonefruit virus research, preliminary studies have demonstrated that leafy *Prunus mahaleb* cuttings show a marked rooting tendency when treated with adenine and indolebutyric acid under conditions limiting cuticular transpiration and affording a supply of certain nutrient materials. Three-in. leafy cuttings were momentarily immersed in a solution containing a wax-based anti-transpirant, 1,000 p.p.m. urea, 5,000 p.p.m. sucrose, 25 p.p.m. thiamine, and 25 p.p.m. nicotinic acid. When dry the bases of the cuttings were soaked 4 hours in 200 p.p.m. adenine, followed by a dip in 3,000 p.p.m. indolebutyric acid in talc and set. One hundred per cent rooting occurred in 3 weeks with an average of nineteen roots per cutting. Similar treatments without adenine produced 40 per cent rooting in 3 weeks and 80 per cent rooting in 6 weeks with an average of 8 roots per cutting. When neither adenine nor indolebutryic acid were used in treatments, no rooting response was demonstrated in 6 weeks. Treated cuttings in all cases remained as fresh as when first taken with no leaf abscission or chlorosis. Similar tests excluding the antitranspirant-nutrient pretreatment brought about death to all cuttings prior to any rooting response.

Apparently continuous variation in tobacco mosaic virus. HAMMACK, MARIAN G. AND J. G. BALD. Physio-chemical and biological differences have been shown to exist between groups represented by ordinary (severe) strains of tobacco mosaic virus and mild strains that appear to be mutants of the severe. In addition, considerable variability of symptom type is possible within each group of strains. Controlled environment may be used to induce variation within an apparently pure strain. In one experimental series single lesion isolates were inoculated into Turkish tobacco plants which were grown in lighted cabinets at 18°C. and 30°C. After 12 weeks the plants were harvested and single lesion isolates, about 20 from each plant, were inoculated into Turkish tobacco plants in the greenhouse. Some isolates from the original severe strains after multiplication in plants at 30°C. were milder than the isolates from plants grown at 18°C. Isolates from the original mild strain multiplied at 30°C. were more severe than those from plants grown at 18°C. A distorting mutant was obtained from plants with the mild strain, grown at 18°C.

Influence of date of collection and distance from pollution source upon foliar fluoride content of apple, cherry, and prune. HENDRIX, J. W., D. F. ADAMS, D. J. MAYHEW, C. J. MANKIN, R. M. GNAGY, AND R. K. KOPPE. Analysis of 373 apple, prune, and cherry samples collected at 2 periods in 1952 from 112 land sections in 4 townships within an air pollution area near Spokane, Washington, revealed foliar fluoride concentrations ranging from 13 to 1,034 p.p.m. on a dry weight basis. The analysis data revealed a relationship between fluoride concentration and 1) plant species, 2) date of collection, and 3) distance from the source of pollution (an alumina reduction plant located within the exposure area). The average foliar fluoride content of apple, cherry, and prune was 194, 185, and 245 p.p.m., respectively. Between July, when the first series of collections was made, and September, when the second series of collections was made, an increase of 43, 104, and 107 p.p.m. was recorded for apple, cherry, and prune, respectively. Proceeding away from the pollution source, the average fluoride content, at 1-mile intervals, was 391, 545, 322, 233, 119, 90, 85, 72, 58, 57, 46, and 41 p.p.m. respectively, for all species combined. The data failed to show a consistent relationship between the fluoride content and degree of plant injury recorded at the time of sample collection.

Studies on the wilt of carnations caused by Pseudomonas caryophylli. HOLTZMANN, O. V., AND W. D. THOMAS, JR. Carnation bacterial wilt, a vascular disease, may be induced by mechanical plugging, a toxin, or a combination of factors. In preliminary experiments carnation cuttings wilted when placed in a filtrate of a bacterial suspension of *Pseudomonas caryophylli* Burkh. Histological explorations of naturally infected carnation plants revealed that the xylem vessels in part were plugged. Lysis of xylem vessels was also observed. Longitudinal stem sections showed that infection was carried to upper portions of a branch and into the leaves. Bacteria were not found in parenchyma tissue, although in some cases there was apparent proliferation of cells in the form of a periderm around a small infected area of xylem vessels. Carnation cuttings suspended in bacterial filtrates in which proteins and other large molecular compounds were removed, did not wilt. Paper-partition chromatography of free amino acids showed significant reductions in the concentration of some of the free amino acids in diseased as compared with healthy carnation plants. Quantitative sugar analysis showed an increase in fructose and a decrease in an unidentified sugar in diseased plants, whereas glucose remained the same. Indications are that wilt-induction is due to combined factors rather than a single factor.

Methods of evaluating severity and control of mint rust. HORNER, C. E. Quantitative analysis revealed that yield losses of peppermint caused by *Puccinia menthae* were due primarily to the destruction of oil glands on leaves by erupting uredinial sori and secondarily to leaf drop. A method of evaluating disease severity consisted of establishing 4 disease classes based on 0, 0–5, 6–25 and 26–50 per cent reduction in oil gland numbers per unit area of leaf sur-

face. Leaves were taken from 3 successive positions on the main stem and were used to establish a standard to evaluate rust severity and control in fungicide plots. A disease index was computed for each plot by the McKinney infection-index formula. There was a high correlation between disease index and yield. The disease index was a more reliable criterion of yield loss and rust control than was leaf drop. Disregarding the possibility of stimulation of plant growth by the fungicides, the computed disease index based on percentage destruction of oil glands gave a value closely approximating the actual percentage of reduction in yield by rust.

Pathogenicity of Verticillium isolates from various hosts on peppermint. HORNER, C. E. Single spore or single hyphal tip cultures of *Verticillium albo-atrum* from 17 sources representing 11 host species were used to inoculate peppermint plants both by wounds and by infested soil. Inoculated plants were grown at a mean greenhouse temperature of 22°C. for 7 weeks. Distances of growth in the stems of soil-inoculated plants were determined by reisolations from points 2 in. below the soil line, at the soil line and at 2, 4, 6 and 8 in. above the soil line. Reisolations from wound-inoculated plants were made at 2 in. below, 2, 4, 6, 8 and 10 in. above the point of inoculation. All isolates grew in mint stems, but typical wilt symptoms were expressed only by isolates originally taken from mint with wilt symptoms. Significant differences were found in the distances isolates grew in mint stems by which the isolates were placed in 3 groups. Group 1 contained isolates originally from mint with severe wilt symptoms in the field. Group 2 contained isolates from mint without wilt symptoms. Group 3 contained isolates from the following hosts: *Acer rubrum, Arctium minus, Chenopodium album, Cirsium arvense, Lycopersicon esculentum, Malva rotundifolia, Paeony albiflora, Rubus occidentalis, Solanum tuberosum* and *Symphoricarpos albus. Arctium minus, Cirsium arvense* and *Symphoricarpos albus* have not previously been reported as hosts for *Verticillium albo-atrum.*

Predisposing effects of air temperature and nitrogen supply upon injury to some herbaceous plants fumigated with peroxides derived from olefins. KENDRICK, J. B., JR., JOHN T. MIDDLETON, AND ELLIS F. DARLEY. Viroflay spinach grown for 10 weeks at a mean air temperature of 13°C., and then at 24°C. 1 week before fumigation with the peroxides of certain olefins, was damaged 4 times as severely as those kept at 13°C. continuously. Plants with abundant nitrogen supply were injured 5–7 times as much as comparable nitrogen-deficient plants. Romaine lettuce grown for 10 weeks at 24°C. and then at a mean air temperature of 13°C. 1 week prior to being fumigated, showed only a trace of injury while those kept at 24°C. continuously had 80 per cent of the leaf area damaged. Endive responded in a similar way. Romaine plants growing at a mean air temperature of 13°C. for fewer than 4 days did not acquire any tolerance to this type of air pollution injury. Barley and oats growing with an abundant nitrogen supply were injured slightly upon fumigation, whereas those grown with limited nitrogen were not injured. Oats appear to be damaged more easily than barley. This study indicates that minimum concentrations of these phytotoxicants necessary to produce plant damage must be considered in relation to the pre-fumigation environment of the plant.

Histological studies of injury produced by a new curly top virus strain in resistant sugar beets. LACKEY, C. F. A new curly top virus strain isolated by N. J. Giddings, which he designated strain 11, was reported by him as injuring the resistant sugar beet SL 68 almost as severely as the susceptible beet SL 842. The variety SL 68 is highly resistant to virus strains previously tested. His virulent strain 1 produces severe injury to SL 842, but does little damage to SL 68. Strain 1 causes severe phloem necrosis but relatively little hyperthrophy in petioles and root tips of SL 842. In contrast strain 11 causes greatest injury in the petioles and very little injury in the root tips. In petioles of SL 842 and SL 68 injury consists of extensive phloem hypertrophy and necrosis. The general effect is more severe than that of strain 1. An SL monogerm hybrid selection showed considerable resistance to strain 11. It produced definite hypertrophy of phloem tissues but there was much less necrosis and bundle disorganization and some phloem tissue was left capable of functioning. Thus, both resistant and susceptible beets reacted to strain 11 by the production of an unusual amount of hypertrophy of phloem tissue. Resistance to this strain seems to consist of resistance to production of phloem necrosis whereas with strain 1 it consists of resistance to production of both necrosis and hypertrophy.

Loganberry rootrot on Vancouver Island. MCKEEN, W. E. For the last few years, loganberry plantations have continued to decline on Vancouver Island, and yields have been consistently below those expected by farmers and agronomists. Loganberry plants appear to develop normally during early spring, but after close examination, it is found that a reduced number of new shoots emerge from the old crown, particularly in the lower regions of the field. Sometimes, new shoots begin to emerge from the soil, but growth suddenly stops, and the shoots become brown and necrotic. Later in the season, when the fruit is beginning to mature, a physiological drought and starvation of the plant seems to occur. When the unhealthy plants were removed in mid-summer, the crown and root system were not necrotic, and new white healthy roots were present, but they were restricted to a relatively small area about the crown of the plant. A great many roots, taken from loganberry tips planted in soil taken from low areas in diseased fields were necrotic and frequently completely decomposed. Tips removed from soil in the field during January and February 1953 very often were diseased. Microscopic observations of many diseased roots showed that a *Phytophthora* species was always present and sporangia were readily observed about the root. Frequently, a *Pythium* species was present with *Phytophthora,* and this hindered the isolation of *Phytophthora.* Occasionally, a nematode belonging to the genus *Paratylenchus* was noticed within the necrotic roots.

Olefinic peroxide injury to bean as influenced by age, variety, chemical additions, and toxicant dosage. MIDDLETON, JOHN T., J. B. KENDRICK, JR., AND ELLIS F. DARLEY. The extent and severity of injury to primary leaves of Pinto bean fumigated with olefinic peroxides increases as the age of leaves increases from 2 to 16 days. Damage to compound leaves of 16-day-old plants is greatest on the oldest and least on the youngest, with none apparent on terminal, unexpanded leaves. The lower surface of primary and older compound leaves is typically glazed and sunken, whereas both surfaces of younger, apical leaves are spotted with small, punctate, reddish-brown discrete spots, ranging from 0.5 to 2.0 mm. in diameter. The extent of injury at a given concentration of toxicant is directly proportional to duration of fumigation. Varieties of *Phaseolus lunatus* and *P. vulgaris* respond differentially to the toxicant. Golden Cluster, Pink, Pinto (gray), Small White, and Fordhook 242 are among the most susceptible, and Bountiful, Pinto (brown), and Fordhook Concentrated are among the most resistant. Leaves may be protected from injury by aqueous sprays of manganese or zinc ethylenebisdithiocarbamate applied immediately before fumigation. Bordeaux does not protect when applied at concentrations of 3-3-100 and 6-6-100 but does when applied at 12-12-100. This suggests 2 mechanisms of protective action: direct chemical influence and altered plant activity.

Bacterial stem rot of Pelargonium hortorum. MUNNECKE, DONALD E. Bacterial stem rot (*Xanthomonas pelargonii*) of *Pelargonium hortorum* is limiting field production of geraniums in southern California. Leaf infections produce either small necrotic spots or necrotic sectors depending upon the variety parasitized. Later the stems rot and the plants die. Roots are intact until death of the plant which aids in distinguishing bacterial stem rot from Rhizoctonia or Pythium stem rots. Infected cuttings rapidly blacken

and rot in rooting media. The vascular-inhabiting bacteria may be transmitted by cuttings from symptomless plants, by rooting media containing infected cuttings, and by knives used in field or packing operations. Field observations indicate rapid spread by rain and overhead sprinkling. Soil carryover in greenhouse experiments was less than 2 weeks. Field experiments are in progress to control the disease by selection of stock plants, field rotation, and sanitation in harvesting and handling of cuttings. Disease-free mother block plants growing in steamed or chemically-treated soil, carefully rogued and handled to prevent re-contamination, will eliminate initial infection. It is hoped that furrow-irrigated plantings of healthy stock plants on rotated land may be kept bacteria-free by dipping clippers in 1:1000 mercuric chloride solution when taking cuttings between plants and by following strict sanitary measures.

Thermal death range of Verticillium albo-atrum. Nelson, Paul E. and Stephen Wilhelm. The minimal lethal temperature in hot water (10-minute exposure) for a rose and tomato isolate of *Verticillium albo-atrum* Rke. and Bert., growing on previously sterilized uniform pieces of flower stalks of *Plantago lanceolata* L. was 47°C. for hyphae and conidia, and 50°C. for hyphae, conidia and microsclerotia. A 40-minute exposure at 47°C. also killed the microsclerotia. Infected rose and geranium cuttings failed to grow after exposures to several temperatures lethal to Verticillium. Microsclerotia exposed in a dry atmosphere at 49°–50°C. survived for 6 months. Conidia in such an atmosphere succumbed within 3 days. Microsclerotia of 2 other isolates survived for 2½ years at 40°C.

Pathogenicity comparisons between Sclerotinia laxa and S. fructicola. Ogawa, Joseph M. Blossoms inoculated with *Sclerotinia laxa* and *S. fructicola* and held at about 20°C. in a saturated atmosphere for 24 hours, then incubated at about 22°C. at about 82 per cent R.H. for 5 days, were read for the number showing blight (necrosis of pedicel or peduncle). No sharp distinctions were found between the 2 organisms in their ability to cause blight in apple and several varieties of almonds, apricots, plums, peaches, and cherries. In general, *S. fructicola* was superior to *S. laxa* in inducing blossom blight; this was especially marked in peaches. Prior to opening of the petals, peaches and almonds were less susceptible to blight than after opening, whereas with plums, apricots, and cherries there was no difference. Both organisms were capable of producing blight when the protruding stigmas of unopened blossoms of Oriole peach and Cranberry plum were inoculated. With Drake almond the stigma inoculations produced the highest percentage of blight. Inoculations in the calyx cups produced high percentages of blight in almonds, apricot, and plums. In inoculations made from May to the following March, the 2 organisms produced twig cankers similar in size on almond, apricot, and nectarine. Largest cankers were produced in the spring and smallest in the summer. In the spring *S. laxa* exceeded *S. fructicola* in length of cankers produced.

Antigenic relationships among strains of tobacco mosaic virus. Rappaport, Irving, and Albert Siegel. A serological investigation has been made on a number of different strains of tobacco mosaic virus. Quantitative precipitin tests were performed with rabbit antisera to determine what antigenic relationships existed among the strains. By means of appropriate absorptions and precipitin analyses, 8 strains of TMV have been classified into 4 groups. The members within a group are closely related to one another, and are more distantly related to the viruses outside the group. This antigenic classification agrees precisely with one obtained in this laboratory based on biological and physical-chemical data. To date, symptomatology on *Nicotiana tabacum* has been the only means available for identifying member viruses within a group. It is now possible to show that subtle antigenic differences exist among group members, which distinguish them from one another.

The effect of various organic compounds on tobacco mosaic virus multiplication. Schlegel, David E. and T. E. Rawlins. Ninety organic compounds were tested to determine their effect on tobacco mosaic virus (TMV) multiplication. Leaf discs were taken from mature lateral leaves of tobacco plants which had been inoculated the previous day. These discs were floated for 6 days on water or on various concentrations of the compound to be tested. The floated discs were exposed to alternating 12-hour periods of artificial light (300 foot-candles) and dark, and the solutions were changed every 24 hours. The virus in each disc sample (usually 15 discs) was purified and the yield determined spectrophotometrically. Discs floated on a 0.01 per cent solution of diazouracil consistently yielded about 70 per cent less virus than discs floated on distilled water; those on 0.01 per cent DL-isoleucine and L-isoleucine solutions yielded about 50 per cent less than those on water; D-isoleucine had no detectable effect on virus yield. One tenth per cent glucose-l-phosphate solution caused 58-97 per cent stimulation in virus multiplication when tested under laboratory light conditions.

The classification of strains of tobacco mosaic virus on the basis of biological, physical, and chemical characteristics. Siegel, Albert and S. G. Wildman. Eight strains of tobacco mosaic virus were analyzed for symptom expression in *Nicotiana tabacum* and *Nicotiana sylvestris*, color of virus pellet when prepared in cacodylic acid buffer, electrophoretic mobility at pH 7, and sensitivity to inactivation with ultraviolet light. The strains were found to fall into 4 groups when judged by the 4 criteria other than symptoms in *N. tabacum*. Within a group the strains are identical in all properties except symptom expression in *N. tabacum*. In contrast, 2 of the strains have the same symptoms in *N. tabacum* although they differ in each of the 4 other characteristics investigated.

A new disease of cultivated blueberry. Stace-Smith, R., Paul H. Wooley, and Edward K. Vaughan. For the past 5 years, a serious stem canker of blueberries (*Vaccinium australi* L.) has been under observation in Western Oregon. The disease begins, usually in January or early February, as a watersoaking, but rapidly changes to definite reddish-brown to black cankers which may extend from a few mm. to the entire length of the cane. Only the canes which are produced the previous season are affected. All buds in the cankered area are killed. Sometimes the stems are girdled, but in other cases the vascular tissues are not damaged, and the buds both above and below the canker grow normally. Plants in the field and cuttings in the propagating beds are attacked. The varieties Jersey, Atlantic, Scammel, Coville, Evelyn, and N51G appear to be most severely affected. Rubel, Pioneer, and Burlington are seldom affected, and the *V. australi* × *V. lamarkii* hybrids Weymouth, June, and Rancocas are highly resistant. A species of *Pseudomonas* has been shown to be the cause of the disease.

Field and laboratory tests of calcium polysulphides and chlorinated hydrocarbons for the control of the cotton root-rot fungus (Phymatotrichum omnivorum). Streets, R. B. Calcium polysulphides were applied in open furrows at the rate of 20, 40, 80, and 160 gal. per acre to land on which 97 per cent of cotton plants had died the preceding year. Two chlorinated hydrocarbons were injected into similar plots with a McLean injector: P-162 at 6-in. depth at rates of 20, 40, 60, and 180 lbs. per acre, and CBP55 at 20 and 30 gals. per acre, at 12-in. depth and at both 6 and 12 in. None of the plots showed control of root rot in cotton. A similar experiment on replicated 1/7 acre plots of alfalfa infested with root rot, in which calcium polysulphides were applied at rates of 35, 70, and 140 gal. per acre in the irrigation water, showed no significant difference in amount of root rot. P162 was injected in 1/12-acre plots in a 5 × 5 Latin square at 4 rates by a tractor-drawn outfit, but no control of root rot was secured in the cotton crop following. Applications on a variety of fruit and ornamental trees and shrubs likewise failed to control root rot. Laboratory experiments gave similar results.

A comparison of two methods for the estimation of virus concentration. TROXEL, A. W. Biological assay is the classical method of estimating virus concentration in plant tissues. The method is subject to many variables some of which cannot be controlled readily. Under ideal conditions and assuming the virus activities to be constant, concentration differences as large as 10–15 per cent can be detected. The ultraviolet absorption method for estimating virus concentration measures both active and inactive virus. Duplicate samples regularly agree within 4 per cent. Therefore the method is better for experiments which give relatively small differences in virus concentration. Significant differences have been detected by ultraviolet where none were obtained by biological assay. Regular differences with the absorption method sometimes yield erratic results with bio-assay. The latter is used, however, to estimate virus activity.

Ringspot-like viruses of rhubarb. VAUGHAN, EDWARD K. AND JOHN W. YALE, JR. Several symptom patterns suggestive of virus infection have been observed on rhubarb (*Rheum rhaponticum* L.) in the Willamette Valley of Oregon. Typical plants, transplanted to pots in the greenhouse, developed different symptoms from time to time; the symptoms varying in both degree and form. The viruses obtained from the plants varied also in their ability to infect other plants. Symptoms on rhubarb are as follows: chlorotic spotting, necrotic stippling, chlorotic and necrotic ring-spots, veinclearing, and a pale green mottle. In the field, they are conspicuous in early spring and become less apparent during the summer. Factors controlling the type of symptoms expressed are not known. The virus was transmitted to Bountiful bean, broad bean, buckwheat, cucumber, curly dock, spinach, Sweet William and zinnia by triturating infected leaf tissue in 0.1 M disodium phosphate and rubbing leaves dusted with carborundum. Symptoms produced on Bountiful bean and broad bean are comparable to those produced on these hosts by known strains of tomato ringspot viruses from cucumber. The symptoms on Sweet William are sufficiently specific for ringspot to place this virus provisionally in the genus *Annulus* until protection and serological tests can be made.

Control of root rot by row application of soil fumigants. WATSON, R. D. Early root rot infections are generally concentrated near the cotyledenary node of peas and the transition zone between root and hypocotyl on beans. This observation suggested that fungicidal fumigants placed at the proper level when the seed was planted might offer effective control. This assumes the fumigant will be nontoxic to the seed or volatile enough to be dissipated before the seed starts to grow and becomes more sensitive to the fumigants. The 2 fumigants tested gave excellent control of root rot in greenhouse and field tests for 2 years. Control with Shell CBP-55 was more consistent than with formaldehyde. Control of root rot was accompanied by improved stands and marked increases in yield of peas and beans. Application equipment was developed which was both simple to make and easily adapted to most planters. The equipment consisted of a constant-head-vacuum arrangement for the delivery tank and the rate of application was controlled by capillary tube or valves in the delivery tubes. The fumigant under gravity flow was applied with the seed in the planter shoe opening.

Pythium ultimum, an injurious component of the rhizosphere in certain soils. WILHELM, STEPHEN. Turkish tobacco grows poorly in some soils in which strawberries have failed and grows well in some other soils in which strawberries have grown satisfactorily. The factors responsible for the poor growth have been eliminated in tests, by autoclaving, fumigating with chloropicrin, exposing to drying for 10 days at 49°C. and 46°C., but not at 37°C., and by additions of large quantities of hoof and horn grist and ammonium sulfate. Soils thus treated permitted 10–12 times as much growth as the untreated soil. *Pythium ultimum* was a consistent component of the root surface microfloras of the poor tobacco plants. It was not present in a field soil which supported good growth of strawberry nor in a soil unfavorable for strawberry after autoclaving, fumigating, or drying. Fumigated field soil artificially infested with *P. ultimum* alone, or in combination with *Fusarium oxysporum, F. roseum,* and an isolate of bacteria, generally gave poor growth of the tobacco. A faint yellow-brown coloration of the roots indicated a parasitic relationship between the tobacco roots and *P. ultimum.* A different species of *Pythium* and species of *Fusarium, Gliocladium, Melanospora, Trichoderma, Trichothecium, Phoma,* and *Mucor,* were commonly present in the rhizospheres of both vigorous and poor tobacco plants.

Attempts to control red stele of strawberry with soil fungicides. WOOLEY, PAUL H. The red stele disease of strawberry, caused by *Phytophthora fragariae,* has become a major problem in some of the better strawberry producing soils in the Willamette Valley in Oregon. Preliminary tests were made to determine the possibility of eradicating the fungus by use of soil fumigants and ordinary fungicides applied to the soil. The chemicals used in the experiments were D-D mixture (1-2 dichloropropene, 1-3 dichloropropane) 1380 lbs/A., nabam 1380 lbs/A., CBP 55 (55 per cent 1-chloro-3-bromopropene) 1490 and 2980 lbs/A., and hexachlorocyclopentadiene 80,100,200,400 lbs/A. Under the conditions of the experiments, none of the materials were effective in controlling the fungus. The investigations are being continued using these and other chemicals as well as different methods of application.

Effects of fungicides and fertilizers incorporated with the soil upon the incidence of beet seedling necrosis. YALE, JOHN W., JR. No significant effect upon the stand of seedlings, yield, or grade of beets was obtained when Ceresan M, Manzate, ferbam, Captan, and C.O.C.S. were incorporated with the soil at 5 lbs. per acre, either alone or in combination with fertilizers. Calcium nitrate mixed with the soil before planting significantly increased the stand of seedlings while ammonium sulphate had no such effect. Greenhouse tests confirmed the beneficial effects of the nitrate fertilizer and showed that treble superphosphate, alone or in combination with calcium nitrate, had no significant effect on incidence of the disease. Potassium chloride and sodium nitrate together increased stands of beet seedlings much more than either chemical alone. Fertilizer treatments were found to be effective only when fresh field soil in normal biological equilibrium was used. Manzate alone at 20 lbs. per acre, and Ceresan M at 20 lbs. per acre with a fertilizer containing sodium nitrate significantly increased stands. The presence of nitrate fertilizer with Manzate completely nullified the effects of the fungicide.

Host adaptation of tobacco necrosis virus. YARWOOD, C. E. When a single lesion strain of tobacco necrosis virus was maintained by mechanical transfer about once a week to fresh tobacco leaves, it was highly infectious (producing many lesions), and moderately invasive (moderate sized necrotic lesions which increased slowly in size) on Pinto bean. When maintained by weekly transfer on bean, it gradually became more invasive on bean, but less invasive and less infectious on tobacco. After being maintained for 22 months on bean, most inoculations on tobacco failed. When the virus which had been maintained on bean was passed through petunia roots, it became less invasive on bean, but when passed several times through bean leaves its invasiveness to bean was gradually restored. When the virus which had passed through petunia roots was transferred to tobacco, a few large relatively nonnecrotic lesions developed. The virus maintained on bean required almost twice as long a period after inoculation to show lesions on bean, as did the virus maintained on tobacco leaves or petunia roots. These results could be explained by mutation (Fulton, 1952. Phytopathology 42: 156.) or by adaptation.